THE STABILITY AND SENSITIVITY OF INSTRUMENTS USED IN SPECTROPHOTOMETRIC ANALYSIS

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Received March 22, 1955

AN important advance in the development of the absorptiometric method of analysis has been made by the recent introduction of differential spectrophotometry by Hiskey and his colleagues[1,2,3]. Various applications of the differential technique have been described[4,5,6], and it is claimed that precision equal to or exceeding that of gravimetric processes may be achieved. The technique differs from that of conventional methods of absorptiometric analysis in that, instead of measuring the intensity of the light transmitted through the solution of unknown concentration against the incident light, the comparison is made with the intensity of light emerging from a solution of the absorbing substance in known concentration. It is also customary in differential spectrophotometry to use solutions of exceptionally high optical density and concentration. In other respects the technique resembles that of conventional methods of absorptiometric analysis and, as Hiskey[2] has pointed out, is subject to a similar limitation, viz., that the precision is determined ultimately by the magnitude of various "uncertainty" factors: chief amongst these are the discrimination, sensitivity or response, and over-all stability of the instrument.

A substantial improvement in discrimination may be effected by replacing the logarithmic optical density scale of the Unicam SP500 spectrophotometer by a linear absorption potentiometer of the type recently described by the writer[7]. By this means the scale-length of the instrument is expanded to such an extent that the discrimination is multiplied by factors of 5, 10, 20 and 25 throughout the density regions 0·0 to 0·2, 0·2 to 0·5, 0·5 to 0·8 and 0·8 to 1·3 respectively. The present communication is concerned with the remaining "uncertainty" factors referred to above, viz., with methods of increasing the over-all stability and sensitivity of the instrument.

The power supplies required for the operation of the amplifier and tungsten lamp of the Unicam spectrophotometer are normally derived from two 6 volt accumulators, which must be changed at intervals of 8 hours and recharged every 24 hours; the stability of the instrument is thus dependent to a high degree on the care which is taken to maintain the batteries. During a demonstration at the Exhibition of the Physical Society in April, 1954, stabilised power for the operation of a Unicam spectrophotometer was obtained from the public electricity supply mains with the aid of a voltage regulator, and it has been suggested to the writer that a description of this device would be of interest. In addition to affording a substantial improvement in zero stability, a stabilised mains unit of this type offers the advantages that the inconvenience and delay occasioned by the frequent changing and re-charging of accumulators are avoided. The degree of stabilisation afforded by the regulator is such that the readings of the spectrophotometer are unaffected by variations of ± 50 per cent. in the voltage of the power supply.

Of the various methods available for increasing the sensitivity or response of a spectrophotometer, that commonly used has the drawback that the setting of the sensitivity control determines the bandwidth which must be used in the examination of a solution of given optical density. The auxiliary sensitivity control described in the present communication provides for a twelvefold increase in the over-all response without affecting the optical performance of the instrument.

THE STABILISATION OF THE SOURCE OF POWER

In preliminary experiments, rectified current derived from public supply mains was stabilised by means of an electronic voltage stabiliser having a self-regulated cathode heater supply[8]. Owing to the high gain of the amplifier incorporated in the stabiliser and the high voltage required for efficient regulation by electronic means, difficulty was experienced in eliminating disturbances of the pointer of the spectrophotometer arising from neighbouring electrical fields or leakage from the high voltage source. It was eventually decided to rely upon a bridge voltage regulator as a means of stabilising the power supply. Regulators of this type are insensitive to external electrical disturbances and, except when high output voltages are required, are considerably more economical in power consumption than electronic stabilisers. A bridge voltage regulator also has the advantage over an electronic stabiliser that its performance remains unimpaired after 2000 hours of service.

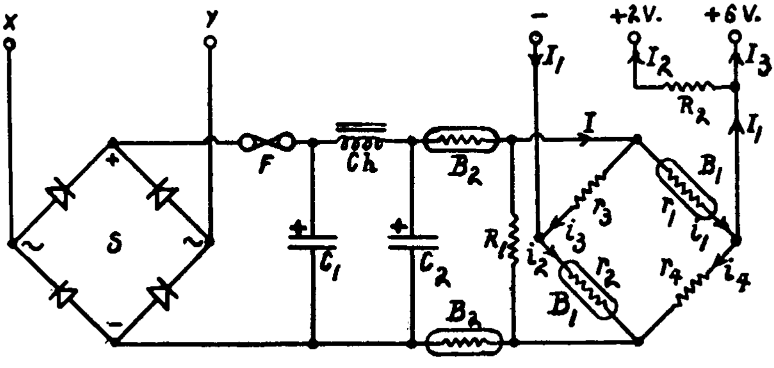

FIG. 1.

The arms r_1 and r_2 of the regulating bridge (Fig. 1) consist of the twin filaments of a Siemens 100 mA. barretter B_1, the bridge being completed by the ohmic resistances r_3 and r_4. As the author has shown[9], a barretter to which a voltage e is applied is electrically equivalent to an ohmic resistance r in series with a constant internal potential v, the value of which may be calculated from the relationship $v = ir - e$, where i is the current flowing through the filament at the applied voltage e, and $r = de/di$ is the differential resistance of the filament. Thus, if $r_1 r_2$ and $v_1 v_2$ are the differential resistances and equivalent internal E.M.F.s of the twin filaments of the barretter B_1, i_1, i_2, i_3, i_4 are the currents through r_1, r_2, r_3, r_4, I is the current supplied to the bridge, I_1 is the output current from the bridge, and r_0 is the resistance of the load, the following relationships apply to the circuit of Fig. 1:—

$$I_1 r_0 = v_1 + i_3 r_3 - i_1 r_1 = v_2 + i_4 r_4 - i_2 r_2 \quad \ldots \quad (1)$$

$$I_1 = i_1 - i_4 = i_2 - i_3 \quad \ldots \quad (2)$$

$$I = i_1 + i_3 = i_2 + i_4 \quad \ldots \quad (3)$$

From these equations it is readily deduced that

$$r_0 i_2 - (r_0 + r_1 + r_3) i_3 + r_1 I = v_1 \quad \ldots \quad (4)$$

and

$$(r_0 + r_2 + r_4) i_2 - r_0 i_3 - r_4 I = v_2 \quad \ldots \quad (5)$$

By eliminating I from (4) and (5) we obtain

$$(r_1 r_2 + r_0 r_1 + r_1 r_4 + r_0 r_4) i_2 - (r_3 r_4 + r_0 r_1 + r_1 r_4 + r_0 r_4) i_3 = r_4 v_1 + r_1 v_2 \quad \ldots \quad (6)$$

If the resistances of the ohmic arms r_3 and r_4 of the bridge are adjusted in such a manner as to satisfy the relationship $r_1/r_4 = r_3/r_2$, equation (6) reduces to

$$I_1 = i_2 - i_3 = (r_4 v_1 + r_1 v_2)/[r_1(r_0 + r_2) + r_4(r_0 + r_1)] \quad \ldots \quad (7)$$

As equation (7) does not contain any term which involves the power supplied to the bridge, it follows that the output current I_1 is independent of fluctuations in the supply voltage.

In order to extend the regulating range, the twin filaments of a Siemens 140 mA. barretter B_2 are connected in series with the input leads to the bridge. As the current passed by the filaments of this barretter at the mid-points of their respective regulating ranges, viz., approximately 128 mA., is somewhat greater than the input current I required by the regulating bridge, it is necessary to connect a resistance R_1 across the input terminals of the bridge in order to bypass the excess current. Alternating current derived from public supply mains is applied, via a step-down transformer, to the input terminals X and Y of a Westinghouse bridge rectifier S, and the rectified output from the bridge S is passed through a filter consisting of the choke Ch and electrolytic condensers C_1 and C_2 in order to remove the ripple voltage. The fuse F is included in the circuit with the object of protecting the rectifier from damage in the event of a breakdown of the electrolytic condensers.

The 3-way cable which emerges from the case of the Unicam spectrophotometer is normally connected to a 6-volt accumulator, the black lead being joined to the negative terminal, the green lead to a 2-volt tapping on the accumulator, and the red lead to the 6-volt positive terminal; the currents flowing along these leads are approximately 76, 14 and 62 mA. respectively. In the circuit of Figure 1 the resistances associated with the regulating bridge are so chosen that an output of 76 mA. at 6 volts is obtained and, in order to ensure that the currents I_1, I_2 and I_3 are 76, 14 and 62 mA. respectively, a resistance $R_2 = 4/0{\cdot}014 = 286$ ohms is connected in series with the +2 V. terminal. The power required for the tungsten lamp of the spectrophotometer is supplied by an additional secondary winding, delivering 6 amperes at 6 volts, on the mains transformer, and the input to this transformer is stabilised by means of a constant voltage transformer. Siemens 100 and 140 mA. barretters have twin tungsten filaments, and their regulating properties are due to changes in the density of the gas surrounding the filaments; in consequence, these barretters are almost completely free from the thermal time lag which is associated with ballast lamps of the conventional iron–hydrogen type.

The stabilisation ratio $\delta V_0/\delta V_i$ of the regulating bridge and series barretter, i.e., the change in output voltage produced by unit change in the voltage V_i across the condenser C_2, depends upon the linearity of the current–voltage characteristics of the barretters and upon the accuracy with which the ohmic resistances in the bridge network are adjusted. The mains unit used at the Exhibition of the Physical Society was constructed by the Doran Instrument Co., Stroud, all resistances being adjusted with an accuracy of ±0·1 per cent. For this unit the value of $\delta V_0/\delta V_i$ was found to be 2×10^{-4}; in other words, a variation of ±1 V. in the voltage across the condenser C_2 gives rise to a change of ±0·2 mV. in the voltage across the 6 V. output terminals. Additional regulation is provided by the constant voltage transformer, and the over-all stability is such that no movement of the pointer of the meter can be detected as a result of changes of ±50 per cent. in the voltage of the supply mains.

Due to the high internal resistance of the mains unit as compared with an accumulator, the dark current and sensitivity controls are in some degree interdependent; if the setting of the sensitivity control is altered to an appreciable extent after the dark current adjustment has been carried out, it will in general be found that the latter adjustment has been disturbed. For this reason, when the mains unit is in use, the sensitivity control should normally be set about 3½ turns from the fully clockwise position before carrying out the dark current adjustment, and should not be disturbed during subsequent measurements.

THE SENSITIVITY OF THE INSTRUMENT

A simplified circuit diagram of the electrical connections of the spectrophotometer is given in Figure 2. The photoelectric current i_p generated by the photoelectric cell P flows through the 2000 MΩ resistor R_2, producing across this resistor a voltage drop E which is applied, in series with an opposing potential derived from the density potentiometer R_{13}, to the grid of the input valve V_1 of a two-stage D.C. amplifier. The measurement consists in adjusting the potentiometer until the output current i_a flowing through the microammeter M attains a predetermined value (300 μA.); the opposing voltage impressed upon the grid of V_1 by the potentiometer R_{13} is then equal to the P.D. across R_2 due to the photoelectric current, and is directly proportional to the percentage transmission of the solution under investigation. The voltage sensitivity $S_v = \delta i_a/\delta E$ of the amplifier is approximately 15 μA./mV., and the current sensitivity $S_i = \delta i_a/\delta i_p = S_v R_2$ is accordingly about 30 μA./μμA.

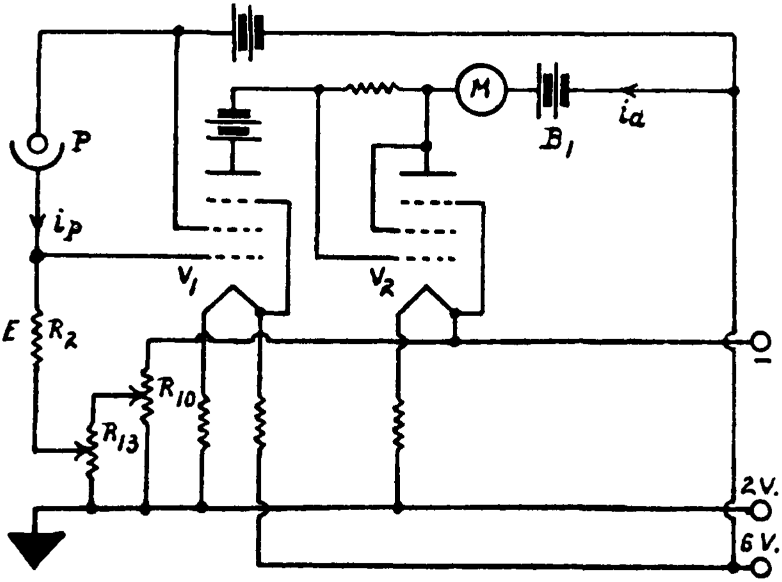

FIG. 2.

No means of controlling the gain or voltage sensitivity is provided, but the P.D. between adjacent subdivisions of the transmission scale of R_{13} may be continuously adjusted between the approximate limits of zero and 14 to 16 mV. by means of the sensitivity control R_{10}, which regulates the current flowing through R_{13}; each of these subdivisions corresponds to 1 per cent. change in transmission. The ratio $S_t = \delta i_a/\delta t$, that is, the increment in the output current i_a produced by unit change in the percentage transmission t, may conveniently be termed the transmission sensitivity; it is evident that if, at a given setting of the control R_{10}, the voltage drop between adjacent subdivisions of the transmission scale is V, the transmission sensitivity is $S_t = \delta i_a/\delta t = V\delta i_a/\delta E = VS_v$. For example, assuming that V may be varied from zero to 16 mV. by adjustment of R_{10}, and that the voltage sensitivity of the amplifier is $S_v = 15$ μA./mV., the transmission sensitivity of the instrument, i.e., the output current obtained for 1 per cent. change in transmission, is adjustable between the limits of zero and $15 \times 16 = 240$ μA. by means of this control.

The sensitivity control R_{10} and the slit width control are interdependent; only one of these controls may be varied at will, the other being a dependent variable. For all normal purposes the manufacturers of the instrument recommend that the sensitivity control should be set to a predetermined value, and that balancing adjustments should be effected by means of the slit width control, which thus becomes the independent variable. Under these conditions the transmission sensitivity is maintained at a constant high level throughout the measurements, regardless of the optical density of the solutions. On the other hand, Neal[5] recommends the use of a constant narrow slit width in differential spectrophotometry on the grounds that variation in bandwidth may lead to interference from other absorbing species present in the solution. When, however, the sensitivity control is used as the independent variable in the manner recommended by Neal, the transmission sensitivity decreases rapidly as the optical density of the solution increases, and the response of the instrument may be reduced to such a low level as to render adequate discrimination impossible.

From the foregoing considerations it is evident that some auxiliary means of increasing the sensitivity without affecting the optical performance of the instrument would be an advantage. An auxiliary control of this nature, known as the "meter sensitivity control", is included amongst the components of the Uvispek spectrophotometer, the provision of this control being facilitated by the use of twin triodes in the output stage. The corresponding stage of the Unicam amplifier contains only 1 valve (V_2 in Fig. 2); nevertheless, the circuit of the amplifier may be modified in a simple and inexpensive manner in order to provide for the addition of a similar auxiliary control.

The microammeter incorporated in the spectrophotometer is a moving coil instrument requiring 600 μA. for full-scale deflection; if this is replaced by a 50 μA. galvanometer, a twelvefold increase in over-all sensitivity is obtained. Inspection of Figure 2 reveals the fact that the meter carries the full anode currents (300 μA.) of the valves; in order that the microammeter may be replaced by a sensitive galvanometer, it is necessary to make provision for a counter current of the same magnitude. The slight alterations in the circuit which are required for this purpose are shown in Figure 3, the counter current i_c being obtained by connecting the stabilised 6 V. power supply, in series with a current-limiting resistor R, across the galvanometer G. As $i_c = i_a = 300\,\mu$A., the appropriate ohmic value of R is $6/300 \times 10^{-6} = 20{,}000\ \Omega$. A high stability resistor of the inexpensive radio type, having a tolerance of ± 1 per cent., is suitable for the purpose. In the circuit of Figure 3, the anode leads of the valves are returned to the negative pole of the 6 V. source instead of to the positive terminal: the voltage tapping on the battery B_1 should accordingly be increased by 6 V. in order to restore the anode voltages to their normal values. A variable shunt (not shown in the diagram) is connected across the galvanometer terminals for use as the auxiliary sensitivity control.

In the modified circuit of Figure 3 the voltage drop across the galvanometer is $i_g R_g = i_c R - E_0 = (i_a - i_g)R - E_0$, where R_g is the resistance of the galvanometer, i_g is the galvanometer current, and E_0 is the voltage (nominally 6 V.) of the source of counter-current; the galvanometer current is thus

$$i_g = (i_a R - E_0)/(R_g + R) \quad .. \quad .. \quad .. \quad .. \quad .. \quad (8)$$

and the increment in galvanometer current produced by unit increase in the photoelectric current i_p is

$$\frac{\delta i_g}{\delta i_p} = \frac{\delta i_g}{\delta i_a} \cdot \frac{\delta i_a}{\delta i_p} = \frac{R}{R_g + R} \cdot Si \quad .. \quad .. \quad (9)$$

As $R \gg R_g$, equation (9) reduces in practice to $\delta i_g / \delta i_p = S_i$.

The circuit arrangement of Figure 3, in addition to providing for a substantial increase in the over-all sensitivity of the instrument, has the advantage that drift of the pointer due to fluctuation in the voltage E_0 of the power supply is further reduced. The change in the galvanometer current produced by fluctuations in the supply voltage is zero when the slopes of the i_a/E_0 and i_c/E_0 curves are identical. From equation (8) it follows that the conditions which must be satisfied in order that the galvanometer current may be zero and may be unaffected by variations in the supply voltage are $R = E_0/i_a$ and $R = \delta E_0/\delta i_a$ respectively. These two conditions cannot be satisfied simultaneously unless, at the normal operating voltage, the tangent to the i_a/E_0 curve passes through the origin of the graph. In practice, this requirement is not accurately fulfilled and, in consequence, perfect compensation for fluctuations in the supply voltage cannot be achieved; nevertheless, a substantial further improvement in the zero stability of the instrument is obtained.

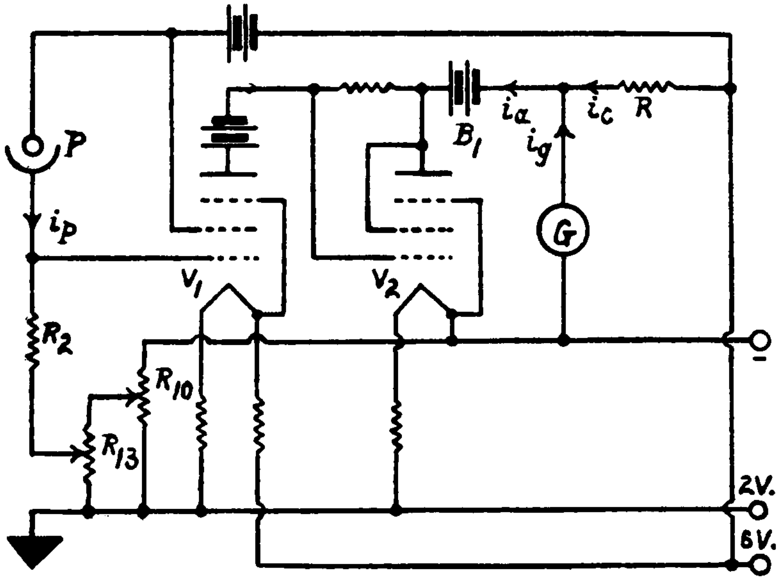

FIG. 3.

SUMMARY

1. With the aid of a bridge voltage regulator the power required for the operation of the amplifier and tungsten lamp of the Unicam SP500 photoelectric spectrophotometer may be derived from public electricity supply mains. The readings of the spectrophotometer are unaffected by changes of ±50 per cent. in the voltage of the power supply, and are also independent of variations in the frequency of alternation of the supply.

2. A description is given of a simple means of effecting a twelvefold increase in the over-all sensitivity of the spectrophotometer, together with a substantial further improvement in zero stability.

The author is indebted to the Royal Society and the University of London for research grants.

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