SOME SENSITIVE AND RECORDING VOLUMETERS

by

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Kinetic measurements in photosynthetic problems often put higher demands for accuracy and speed on the gasometric method used than is fulfilled by standard manometric equipment. In the course of years the present author has used several types of volumeters; some of these have proved to be quite satisfactory, and easy to construct, calibrate and operate.

In general principle a reaction- and a compensation vessel are interconnected by a capillary containing an index droplet. The index is used as zero indicator. At the moment a reading is taken, the gas exchange in the reaction vessel is compensated by a change in volume of the compensating vessel. A mercury delivery screw is turned until the drop is back in the original (zero) position and the displacement of the piston is read. Be V_c and V_r the gasvolumes of compensation and reaction vessel respectively and P_0 the initial pressure in both vessels. Suppose an amount of gas xP_0 is evolved in V_r, after which the index is readjusted by turning the screw h units, each unit representing a volume displacement of $m\mu$l. Then, if P_1 represents the final pressure in both vessels:

$$(V_r + x)P_0 = V_r P_1 \text{ and } V_c P_0 = (V_c - h.m)P_1$$

which yields, neglecting a term xhm:

$$x/V_r = h.m/V_c \tag{1}$$

If the reacting material is suspended in a liquid phase of a volume V_f, leaving a gasvolume V_g in the reaction vessel and if α represents the Bunsen absorption coefficient of the gas studied at the prevailing temperature T, the volume of the gas evolved at a pressure P_0, reduced to 0°C:

$$x = h \frac{m}{V_c} \left(V_g \frac{273}{T} + \alpha V_f\right) \tag{2}$$

COMPENSATION VESSEL AND DELIVERY SCREW

The limit of useful *relative* sensitivity in gasometric apparatus can be expressed as the ratio of the minimum measurable amount of gas evolved x_{min} to the total amount of gas present in the reaction vessel: V_r. This ratio is mainly limited by temperature

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fluctuations*. Dependent on the properties of the thermostat bath (and to a lesser extent on the symmetry of reaction and compensating vessel) a value of $x_{min}/V_r = 1:10^4$ to $1:10^5$ can roughly be taken as attainable in routine apparatus.

If m_{min} represents the smallest volume change to be read on the delivery screw, according to (1) this same ratio can be chosen for the term m_{min}/V_c. Normal micrometer-heads can be used as mercury delivery screws and significantly read down to one division (1/100 mm, ~ 0.3 μl) or less. The volume of the compensating vessel can therefore be 10 to 50 ml. This same combination may be used independently of the volume of the reaction vessel and the latter's size and shape can be chosen as demanded by experimental conditions.

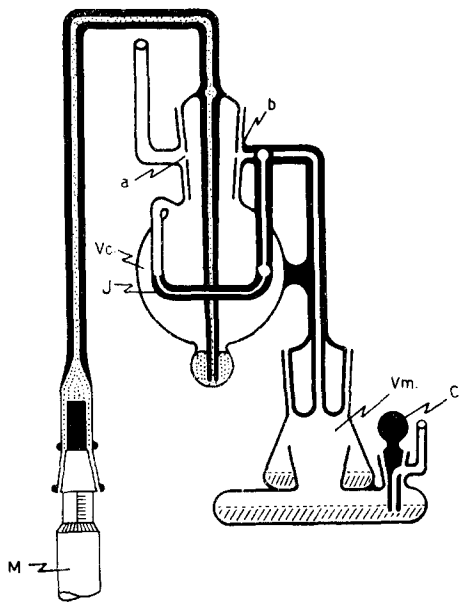

Fig. 1. Differential volumeter. M: micrometer head. V_c: compensating vessel, V_m: reaction vessel, J: index capillary (ø 0.3 to 0.5 mm, length 20 to 50 mm). Holes a and b can oppose glass tubes as shown in the drawing. By means of stopper C, V_c and V_m can be equilibrated with gas mixtures. Turning the whole vessel 90° will close a and b and measurement can start after C is closed.

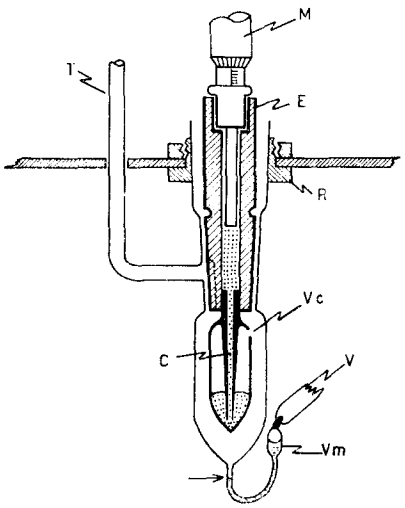

Fig. 2. Differential volumeter. Micrometer head M is sealed into a lucite extension piece E. Into the other end of E is sealed a glass capillary C, which is connected to a mercury reservoir (*ca.* 3 ml). In the lower tapered part of E a groove is cut, allowing for the connection of the compensating vessel V_c with tube T (a stopcock arrangement could be used instead). V_m: reaction vessel, V: metal rod, connected to vibrator—a piece of rubber tubing slipped over its end may be used with fragile vessels.

When filling the screw with mercury, entrapped air should be carefully removed. If it is used upside down as in the arrangement shown in Fig. 1, there will be no leakage as long as an excess pressure is maintained on the mercury (a few cm mercury suffices). In this arrangement a glass capillary connects screw and compensating vessel and the whole apparatus can be mounted on a standard manometer assembly if desired. The design shown in Fig. 2 is more compact and can be used with any type of compensating vessel. Here the delivery screw is combined with its mercury reservoir to form a removable unit. A mercury seal must be present on top of the ground spindle passage (*i.e.* inside the screw) and no leakage will occur even if the apparatus is evacuated.

* B. KOK, G. W. VELTKAMP AND W. P. GELDERMAN, *Biochim. Biophys. Acta*, 11 (1953) 7.

VOLUMETER OF HIGH RELATIVE SENSITIVITY

The volumeter illustrated in Fig. 1 was used in various modifications for accurate measurements of small gas exchanges in large or complicated reaction vessels. A few features of the index capillary combine to simplify the operation of this apparatus:

Only the middle part of the capillary is straight and horizontal; here the zero position of the drop is marked on the glass wall. Towards both ends the capillary has a smooth vertical bend. This adds to the sensitivity of the horizontal part an extension of the usable range: as soon as the gas evolution pushes the drop around the bend, its weight opposes further movement (*i.e.* it shifts towards manometric action). The capillary is suddenly, but smoothly expanded at both ends. This is very helpful in preventing the drop from escaping while placing the volumeter in the thermostat or inadvertently allowing too great pressure differences to develop. An excessive pressure change only breaks the drop at the expanded place, after which it is easily restored to its original position by moving the screw back and forth. In this way, one and the same drop (*e.g.* valeric acid), can be used for a number of days. Smooth movement of the index requires regular cleaning and especially drying of the capillary. Although shaking was usually interrupted during the readings (taken at 1′ intervals), it does not disturb the action of the index drop.

The apparatus is entirely submerged in the bath, and the index can be read through a hole in the insulation of the front wall of the tank. An extended microscope eye-piece mounted on the assembled volumeter is helpful in taking the readings.

The reaction vessel shown in Fig. 1 has two large compartments. The upper compartment holds up to 3 ml of gas absorbing or buffering agents, the lower one 5–10 ml of cellular suspension. To prevent splashing, circular shaking (350 R.P.M., 8 mm stroke) was used with this vessel.

VOLUMETER OF HIGH ABSOLUTE SENSITIVITY

For a given relative sensitivity (x_{min}/V_r cf. (1)) of volumetric apparatus, the smaller the gas phase V_r used per amount of material to be studied, the smaller will be the absolute amounts of gas which can be measured. (For photosynthetic measurements with thin suspensions it is primarily important to realize a small ratio of V_r to the irradiated surface of the vessel.)

For such studies, in which only one liquid phase is to be used in the reaction vessel, this decrease of V_r was realised to the extreme in the designs shown in Figs. 2 and 3.

A small fraction of the suspension liquid itself serves as index fluid (the zero position of the meniscus is indicated by an arrow). With not too small reaction vessels it is possible to keep the amount of suspension fluid in the capillary within one or a few percent of the total liquid phase.

In this apparatus the gas phase is an air bubble, the volume of which can be adjusted as desired by

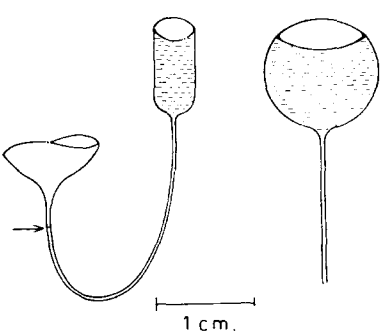

Fig. 3. Reaction vessel and index capillary of medium size (0.3 ml). The capillary is thin walled, inner ø 0.25 mm, outer ø 0.35 mm, length *ca.* 40 mm.

filling the vessel with more or less suspension fluid. Good performance was obtained with a gas phase as small as 1/6 the total volume of V_m. High sensitivity and favourable ratio's of V_r to illuminated area (see above) can be attained in this way.

The index capillary connecting V_c and V_m is rather thin walled; this allows small displacements of the reaction vessel.

A 50 or 60 cycle vibration is induced in this vessel, which causes intense mixing of fluid and gas phases. For this purpose a suitable electric razor or hair clipper can be easily adapted. A small extension rod, fixed to the moving part of the vibrator, touches the vessel or a glass rod sealed onto it. In order to have smooth performance of the index meniscus — of special importance when it is to be automatically recorded — too intense a vibration ought to be avoided. An example of the equilibration times obtained can be found in Fig. 6.

Operation of the volumeter

With the aid of brass ring R (Fig. 2), sealed onto the extension of the compensation vessel, the apparatus can be firmly mounted. Once in its desired position, it need not be removed, since all operations of cleaning and filling can be simply performed through tube T and the opening of V_c.

To fill the reaction vessel with the desired contents, the amount of suspension fluid to be used is pipetted into the compensation vessel on top of the capillary. After greasing its joint, the screw and mercury reservoir unit is placed in position, still leaving tube T connected to the vessel. A two way stopcock connects this tube to either an aspirator or to a reservoir, containing the desired gas mixture. First the stopcock is turned to the aspirator and after the apparatus is evacuated, the stopcock is turned to connect the apparatus with the reservoir containing the gas mixture. The inflowing gas pushes the suspension liquid into the reaction vessel. After a few seconds the index fluid is moved until an unbroken fluid column is obtained with the meniscus in the desired position. For this purpose a syringe, temporarily connected to tube T by means of a piece of rubber tubing, is helpful. The tube T is now closed off by turning the screw unit and measurement started when temperature equilibrium is attained.

The fluid is removed from the reaction vessel by applying suction through a piece of glass tubing fixed in a rubber stopper, which fits on top of V_c after removal of the screw unit. On its lower end this glass tube is fitted with a piece of rubber tubing, which closes off the opening of the capillary. Washing and cleaning of the reaction vessel is done in the same way.

In view of the rather poor index properties of water, the capillary has to be kept clean. A solution of potassium nitrate in strong sulfuric acid was applied over-night between experiments (*i.e.* after removal of the screw unit and preliminary cleaning of joint and vessel).

It generally is good practice to fill the reaction vessel in such a way that the total gas exchange to be measured in an experiment can be compensated by less than one full discharge of the delivery screw; if this amounts to about 1 ml mercury and if V_c is 10 to 20 ml, this represents a relative pressure change as high as 5–10%, sufficient to make corrections necessary. But larger exchanges can eventually be measured by opening T, turning the screw all the way back and restoring the meniscus with the aid of a syringe on tube T.

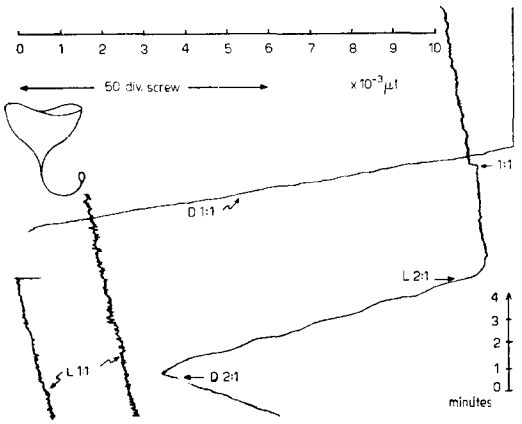

Fig. 4. Recordings made with the smallest reaction vessel used so far ($V_m = 8$ µl, $V_g = 2$ µl). Calibration of the recorder paper both as number of screw divisions and as µl volume change are given for "gear ratio" 1:1, yielding twice the sensitivity of the also used gear ratio 2:1. Gas phase: air plus 2 % CO_2. *Chlorella* cells, suspended in 6 µl carbonate buffer mixture, were exposed to either darkness (D) or a light intensity (L), which slightly over-compensated respiration. The "waves" observable in recording D 2:1 — L 2:1 correspond to small displacements of the vessel for each 180° turn of the screw. This was due to the fact that a mounting, less firm than the one shown in Fig. 2 was used for this recording and for the one given in Fig. 5.

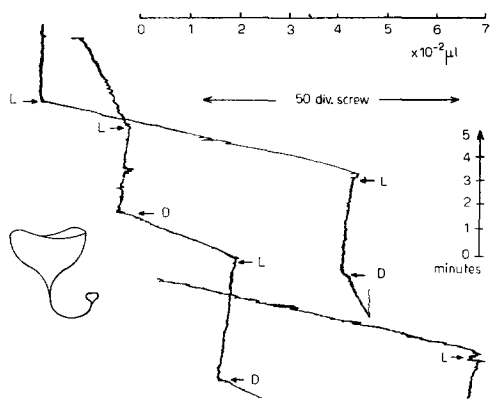

Fig. 5. Recordings made with a reaction vessel of 41 µl ($V_g = 11$ µl). Exposure of *Chlorella* cells in 30 µl carbonate buffer medium to darkness (D) and various light intensities (L). Small irregularities in the recordings are due to breaking of bubbles in the suspension.

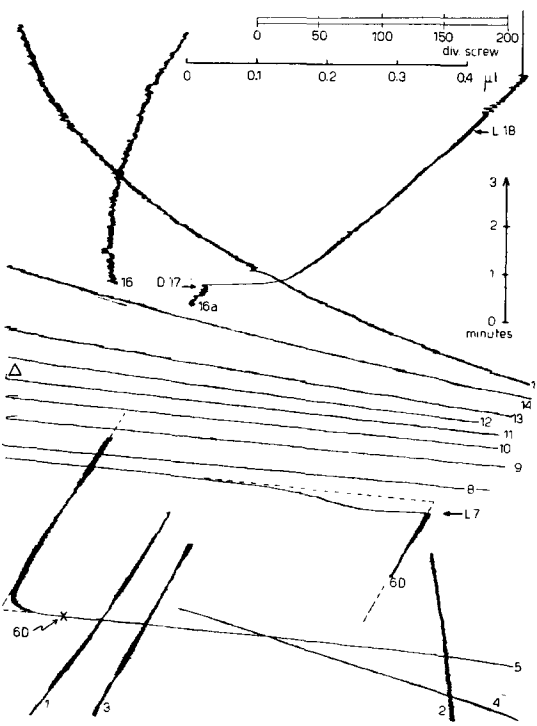

Fig. 6. An experiment made with the arrangement shown in Figs. 2 and 3. Chart speed was 25.4 mm per minute, *i.e.* twice as fast as used for expts. shown in Figs. 4 and 5. Also a different screw was used. $V_m = 290$ µl, $V_g = 60$ µl. Top right an example of calibration in screw divisions is shown: The system was manually moved over 200 screw divisions (4 turns) first to the right, thereafter to the left. Intervals of 50 divisions were marked on the paper. Backlash appears to be virtually absent. In this experiment *Chlorella* cells, resuspended in 230 µl carbonate buffer mixture, were first alternately exposed to darkness (1, 3, 6) and various light-intensities (2, 4, 5). A prolonged exposure to very intense light was then given at the moment marked L7. Each time the pen had travelled all the way across the recorder paper screw SS (see Fig. 7) was loosened and the pen relocated at the right side of the paper (8–16). The strong light caused a progressive decrease of the rate of oxygen evolution, which finally became negative and irregular (15, 16). After darkening (D 17) oxygen uptake became smooth again. At Δ the delivery screw was turned all the way down, after restoring this (see page 40) the recordings were continued (12). The fast equilibration due to vibrating the vessel may be noticed at the start of 6 and 7. The sudden volume change upon addition and removal of strong light (L7, D17) is due to heat effects.

Performance

Size and shape of the reaction vessel may vary greatly. Equally good results were obtained with V_m varying between 3 ml and 8 μl. This is illustrated in the recordings shown in Figs. 4–6.

In the three experiments shown the amounts of cellular material present per unit volume and per unit irradiated area were quite different. In the experiment shown in Fig. 4, 0.18 μl of *Chlorella* cells were used, which represented about 4 μl per cm^2 irradiated area. In respect to light absorption this may be considered as a "thin" suspension, but the dark respiration of this sample ($\sim 3 \cdot 10^{-3}$ μl/min) caused a relative volume change as high as 0.15% per minute. After illuminating the cells, an evolution of about 10^{-4} μl O_2 per minute was observed.

In the experiment shown in Fig. 5 only 0.04 μl of cells were used ($\sim$ 0.02 μl/cm^2) dark respiration ($7 \cdot 10^{-4}$ μl/min) could still be measured accurately within a few minutes.

In the experiment shown in Fig. 6, 2 μl cells were used ($\sim$ 2.5 μl/cm^2). For this type of experiment either a smaller amount of cells or a smaller liquid phase should have been used because the screw was turned all the way down in the middle of an exposure.

The two smaller reaction vessels used for these recordings were sealed onto the same compensation vessel. Both were made, calibrated and tested (see Figs. 4 and 5) within one day, which illustrates the simplicity of the method.

RECORDING VOLUMETRY

The high sensitivity and the short equilibration times characterizing volumeters such as are described in the previous paragraph, readily allow the collection of large numbers of observations. To take full advantage of these features we examined methods for continuous registration of the gas exchange. A relatively simple apparatus was finally used, which optically monitored the index meniscus and registered the turning of the screw. This in no way interfered with the sensitivity of the gasometric apparatus. The system can be used more generally to record any type of measurement, in which a fluid meniscus serves as a null detector.

Monitoring of the meniscus

A narrow, intense light beam ($\varnothing \sim 1$ mm) from a 6 V–0.5 A automobile lamp is concentrated on the capillary bore (see Fig. 7). There is a marked difference in the amount of light scattered sideways by the bore, whether filled with liquid or with air. A fraction of this 90° scattered light is guided towards a photo multiplier cell by a length of glass rod. Figs. 7 and 8 further illustrate this arrangement, which operates as follows:

At the beginning of each experiment the index meniscus is located close to its zero position with the aid of a syringe (see page 38), after which the vessel is closed.

As soon as the fluid level in the capillary rises, the photocurrent decreases. Through a suitable circuit this decrease actuates a relay or a clutch, that reverses the direction of rotation of a motor, which drives the delivery screw. This pushes back the fluid meniscus until enough light reaches the photocell to again reverse the rotation of the motor. This causes the meniscus to oscillate within narrow limits about its zero position. If a gas exchange occurs in the reaction vessel, the delivery screw automatically compensates

for it. Since balancing occurs between more and less light rather than between light and darkness, a better action will be obtained the closer the lower intensity approaches darkness. Stray light, therefore, has to be screened off and the lamp run on a battery or stabilized a.c. voltage.

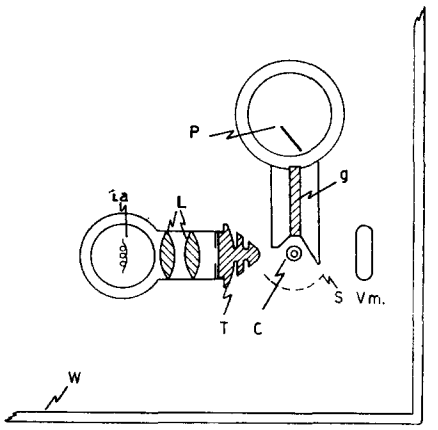

Fig. 7. Arrangement to record the position of the index meniscus. V_m: reaction vessel, C: index capillary. La: lamp, L: lenses (ø 1 cm, f 3 cm). T: lucite tip sealed onto brass tube; incisions are made in it and both outside and inside are painted black except for small circles in both centers. This serves to produce a restricted parallel light beam. g: glass rod (ø 2 mm, length 3 or 4 cm) guiding scattered light towards photocell P. To screen off stray light, the glass rod is sealed in a hole, drilled through a brass or bakelite strip fastened onto the photocell container. S: small screen, fixed onto a rod, extending through the top plate. It can be lifted to make the capillary visible through the thermostat wall W.

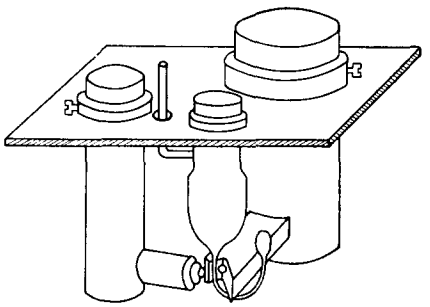

Fig. 8. From left to right: lamp housing, volumeter, photocell housing. All parts are firmly but adjustably mounted on the top plate as were all other thermostat accessories and the vibrator, the tip of which protruded through a hole in this plate. A small, glass-walled thermostat bath (25 × 15 × 15 cm) was mounted to be easily removable underneath the top plate.

Registration

A straightforward and reliable arrangement to make a record of the movement of the screw was built with the aid of a single-pen Brown recorder and is described in detail:

Fig. 9 shows a simple circuit, in which the changes in photocell current actuate the recorder *via* a cathode follower. The amplifier terminals are disconnected from the potentiometer circuit and are used directly, but no further alterations in the recorder have to be made. The recorder motor develops enough power to actuate a mercury delivery screw in addition to the pen carriage and by mechanically coupling both a reliable Servo system is obtained. This is done most simply (though with some backlash) by interconnecting the motor pinion and the screw with a length of flexible (speedometer) cable passing through a hole in the back wall of the recorder. With the aid of a set screw (see Fig. 10),

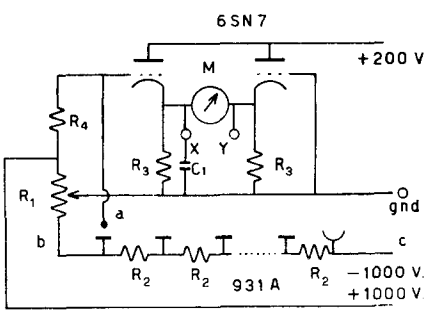

Fig. 9. Photomultiplier circuit. R_1, R_2: 50 kΩ, R_3: 2 kΩ, R_4: 3 mΩ, C_1: 25 to 100 μf. M: 0–5 mA midpoint zero. The photocell and its resistance network (R_2) are mounted in the brass container and connected with a 3-wire cable (a, b, c) with further equipment. x–y: recorder amplifier input terminals. Voltages are to be stabilized and ac components carefully removed (C_1). Meter M is helpful as a control for aligning purposes and for adjusting the meniscus to its zero position before the vessel is closed.

this cable can be easily connected to or disconnected from the micrometerhead. Before each run it may be disconnected and the pen located on a suitable spot on the paper (this can be done either by hand or by motor movement monitored by turning the screw). Connection is then restored and the recording started.

For obtaining optimal performance and cleanest recordings it is useful to minimize backlash in the connection between pen and delivery screw. The use of flexible cable can be restricted to a few ($\sim$ 5 cm) lengths by using instead guided metal rods and devices such as are illustrated in Figs. 10 and 11. If the screw is moved sufficiently slowly, the system is perfectly damped and the pen draws a straight line. It proved to be advantageous, however, to keep the index meniscus slightly oscillating, probably since good wetting of the capillary is maintained in this way. The best performance was obtained by using the 27 RPM Brown motor, either directly coupled to the screw or geared down two- or threefold.

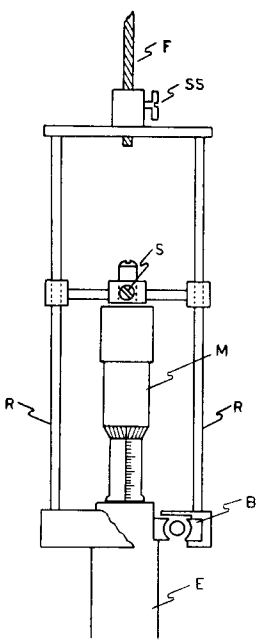

Fig. 10. Accessory to minimise the length of flexible cable used. A cross bar fastened with S onto the micrometer M is free to slide in vertical direction along two bars R, but will transmit circular movement induced *via* cable F, fastened with set screw SS. Ball bearing B, carrying the accessory, fits loosely on the extension piece E. Its inner ø is chosen sufficiently wide so that after S and SS have been loosened the accessory can be lifted up freely and removed.

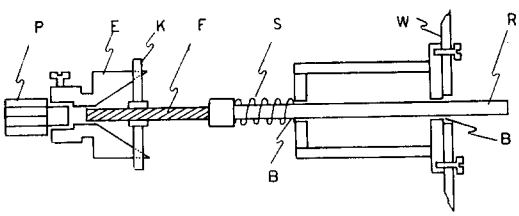

Fig. 11. Arrangement to connect the recorder motor with the delivery screw: Axle R (ø 5 mm) fits loosely in its bearings B, attached to the back wall W of the recorder (the amplifier case has to be moved upwards about 1 cm along this wall). Spring S presses the axle towards the motor. Its end is a 5 cm length of flexible cable F, on which in turn a "knife" K is attached. The latter snaps into a sleeve in the brass extension piece E fixed on the motor pinion P. This extension piece is conical inside, which serves to guide the tip of the axle (protruding *ca.* 1.5 cm beyond the knife) towards its centre. In this way the recorder mechanism can be freely swung out on its hinges; after it is swung back again the mechanical coupling is easily restored.

Calibration

Calibration is done by moving the mechanism and reading the corresponding numbers of divisions on the screw and on the recorder paper. Using this ratio and formula (2), the latter can be simply expressed in microliters gas exchange.

When widely diverging rates of gas exchange are to be measured, it may be advantageous to have a choice of sensitivities available, *i.e.* a number of ratios of screw divisions to paper divisions. This can easily be realised by arranging a set of interchangeable gears between the pen movement and the delivery screw. Such a gear train can be mounted either outside the recorder between motor and screw or inside the recorder between motor and pen movement. The second arrangement, though a little more complicated, is to be preferred, since a change of the ratio does not imply a change in speed

of movement of the screw and therefore leaves the damping of the system unaltered. In our apparatus we removed the motor pinion and remounted it on a separate axle again to drive the pen carriage. The latter axle was connected with the motor axle through the gear train, which was mounted between two brass plates and screwed onto the recorder chassis. A set of change gears ("Meccano" parts) allowed a 9-fold variation of the scale span.

Performance

The performance of the described system controlling the level of the index meniscus surpasses that of visual and manual operation, even if aided by a microscope. For instance, we may give some data as observed with an apparatus, described in Fig. 3: 27 RPM motor, capillary bore ⌀ 0.28 mm, V_c: 14,2 ml, V_m: 290 μl, V_g: 60 μl, one screw division (0.01 mm) representing a displacement of 0.36 μl mercury and a change in $V_g = 1.5 \cdot 10^{-3}$ μl. Reversal of the direction of the motor movement was induced by turning the screw < 0.2 division; this represents a relative change in volume of one part in 10^5 and a displacement of the meniscus of about 5 μ. In actual operation the continuous oscillations were within one or two divisions of the screw, which represented volume oscillations in $V_g < 3 \cdot 10^{-3}$ μl and movements of the meniscus < 50 μ. Arranged in such a way that the full scale of the recorder (100 divisions = 280 mm) corresponded to 430 screw divisions (8.5 revolutions) and therefore to 0.65 μl, short and long term zero constancy with normal shaking intensity of the reaction vessel (observed during an hour) was within one division of the recorder.

The fluctuations within this limit were correlated both in time and in magnitude with the on-off action of the thermostat heater. When the scale span was decreased 9-fold (to 0.072 μl), these temperature fluctuations appeared accordingly increased in the recordings. This indicates that the thermostat rather than the recording system limited the sensitivity; in this case to $\Delta V/V = 1 : 10^4$ (cf.[1]). Slight short term irregularities in the mixing of gas and fluid phases may sometimes appear in the recordings, but do not influence the accuracy of the measurement.

A few records obtained with reaction vessels of different size are given in Figs. 4, 5 and 6. Full scale deflection of the recorder (280 mm) was in the order of 0.01, 0.1 and 1.0 μl respectively. The recording system can of course be used in connection with any other type of gasometric apparatus. Shaking or vibration of the reaction vessel in such cases is best arranged as a well-aligned circular movement, centered around the axis of the index capillary.

SUMMARY

A few types of volumeters of simple construction and operation, characterized by high sensitivity (both absolute and relative) are described. A Servo-system for automatically recording gas exchanges occurring in such volumeters was constructed. Full scale span of the recorder used could be arranged to represent from 10^{-2} μl upwards, with an uncertainty in the order of one percent of full scale. The recording system can be generally used for applications in which a fluid meniscus serves as a null detector, the sensitivity amounting to a few μ displacement.

RÉSUMÉ

Quelques types de volumètres de construction et d'emploi simples, caractérisés par une grande sensibilité (à la fois absolue et relative) sont décrits. Un servo-système destiné à l'enregistrement

automatique des échanges gazeux dans ces volumètres a été construit. L'échelle de l'enregistreur utilisé a été agencée de façon à pouvoir indiquer des volumes depuis 10^{-2} μl, avec une précision de l'ordre de un pour cent de l'échelle totale. Le système d'enregistrement peut généralement être appliqué à des cas où ménisque liquide sert de détecteur de zéro, la sensibilité permettant de percevoir un déplacement de quelques μ.

ZUSAMMENFASSUNG

Einzelne Typen von Voluminometern einfacher Konstruktion und Bedienung, die sich durch ihre hohe absolute und relative Empfindlichkeit auszeichnen, werden beschrieben.

Ein automatisches System zur Registrierung des Gasaustausches, der in einem derartigen Voluminometer stattfindet, wurde konstruiert.

Der Apparat konnte so gebaut werden, dass er bei Vollausschlag 10^{-2} mm^3 oder mehr mit einer Unsicherheit von höchstens 1 % angibt.

Dieses registrierende System, dessen Empfindlichkeit wenige μ Wegdifferenz beträgt, kann man ganz allgemein dann gebrauchen, wenn ein Meniskus als Nullinstrument dient.

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