ON DIFFERENTIAL MANO- AND VOLUMETRIC METHODS*

by

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Numerous biological processes are accompanied by an exchange of gases and the use of manometric methods in studying those reactions is widespread at present.

Next to the few types of instruments in common use, several variations adapted to special purposes have been described, *cf.* DIXON[1], GLICK[2].

In principle the method is very simple and this may be the reason why scarcely any attention has been paid to its fundamental limits.

For kinetic experiments in the field of photosynthesis we are in want of an apparatus as accurate and sensitive as is consistent with routine management and simple construction.

To this purpose some preliminary experiments were made and the results discussed. A brief report here of is following.

INTRODUCTION

In Fig. 1 a differential respirometer is represented. Be: the initial pressure in both vessels P_o (in length of index fluid), their volume up to the meniscus of the index fluid in the symmetrical position $V_c = V_m = V_o$, α the slope of the manometer legs as indicated in the figure, c the capillary cross section and x the distance travelled by both menisci after an evolution of a gas volume V_e (of pressure P_o) in V_m.

Then approximately $\left(\text{neglecting higher powers of } \dfrac{cx}{V_o}\right)$

$$V_e = 2x\left(\frac{V_o}{P_o}\sin\alpha + c\right)$$

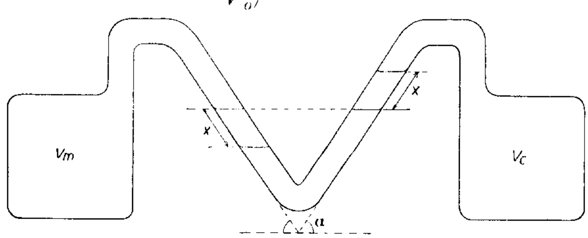

Fig. 1. Differential respirometer.

When choosing $\alpha = 90°$ a "manometer" is obtained for which holds (provided as usual $c \ll V_o/P_o$) $2x = V_e \cdot P_o/V_o$. Using a "volumeter" in which $\alpha = 0°$ and x is read: $x = V_e/2c$ (1).

The sensitivity: $2x/V_e = P_o/V_o$ of a manometer increases with decreasing V_o (P_o being constant). In practice, however, beyond a certain limit it becomes difficult to decrease V_o without causing a

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proportional decrease of V_e, since the latter is determined by the amount of reacting material in V_m.

When other factors have to be considered for the construction and filling of V_m (*e.g.* light absorption by photosynthesizing cells) the volumeter at first sight offers advantages, since the sensitivity $x/B = 1/2c$ increases independently of V_m, when c is chosen smaller.

When using kathetometers or small capillaries both methods theoretically can be made very sensitive. We will now discuss the factors which are limiting in practice and the extent to which they interfere.

Movement of a fluid column in a capillary

A drop of fluid was placed in a glass capillary. By giving the capillary an inclination the drop started to move. The speed of movemeut (u in mm/sec) was measured. The effective pressure applied was computed from the angle of inclination, weight of the drop and capillary cross section. Individual estimations of u varied up to 10% and therefore the average of 10 readings was used for each set of conditions.

Effective pressure (ΔP in mm H_2O), capillary diameter (d in mm), length of drop (l in mm) and type of fluid (viscosity η in c-poises) were systematically varied, which led to the following results:

Fig. 2 shows a linear relation (holding within $\pm$ 4%) between u and ΔP: $u = f\Delta P$, f depending on various factors.

The curves in Fig. 2 do not exactly pass the origin, this effect is discussed below.

The relation between f and l is shown in Fig. 3, inverse proportionality between f and $\sqrt{l}$ is evident: ($f = \beta l^{-0.5 \pm 0.1}$).

In Fig. 4 $f\sqrt{l}$ is plotted against d and the proportionality to $d^{1.5 \pm 0.2}$ is found.

Fig. 5 indicates inverse proportionality between η and $f\sqrt{l/d^3}$, holding for various organic fluids with approximately equal capillarity constants. The exceptional behaviour of pure water can be explained by its relatively high surface tension.

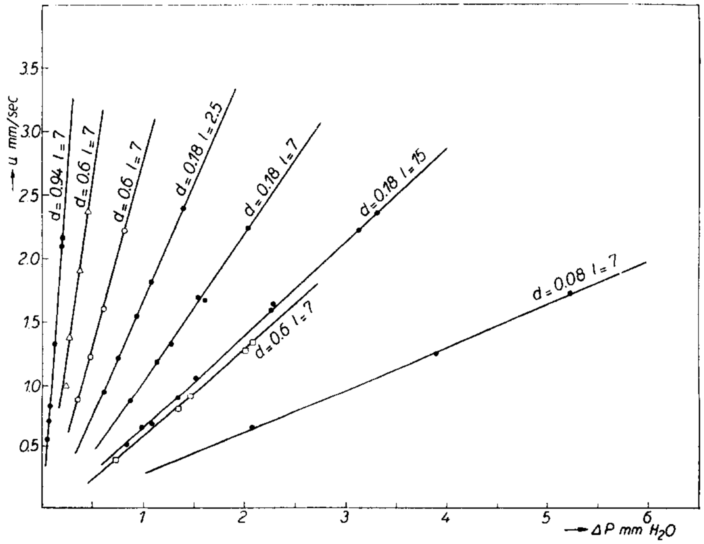
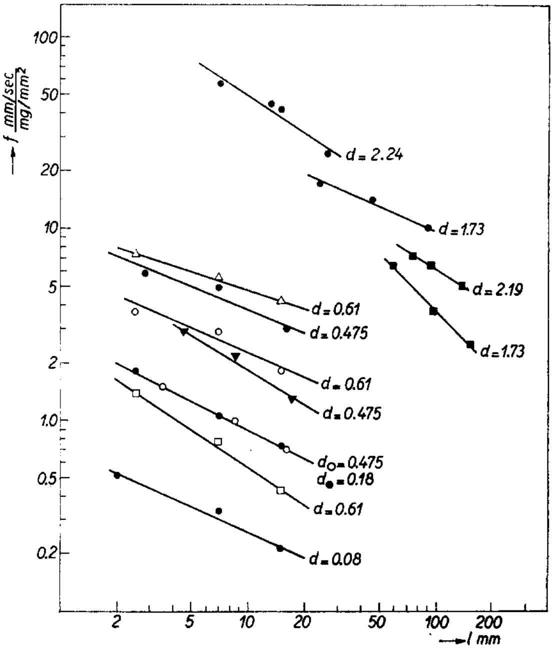


Fig. 2. Relation between u and ΔP. d and l in mm. Various fluids indicated as in Fig. 3.

Fig. 3. Relation between f and l. ● Xylol, △ Decane, ○ *Iso*valeric acid, □ Diethylphthalate, ■ Water, ▼ Kerosene.



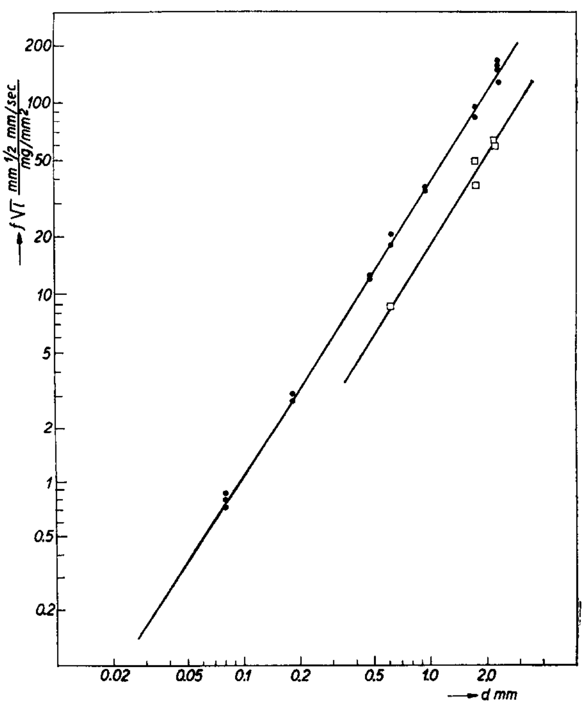


Fig. 4. Relation between $f\sqrt{l}$ and d for Xylol (●) and Water (■).

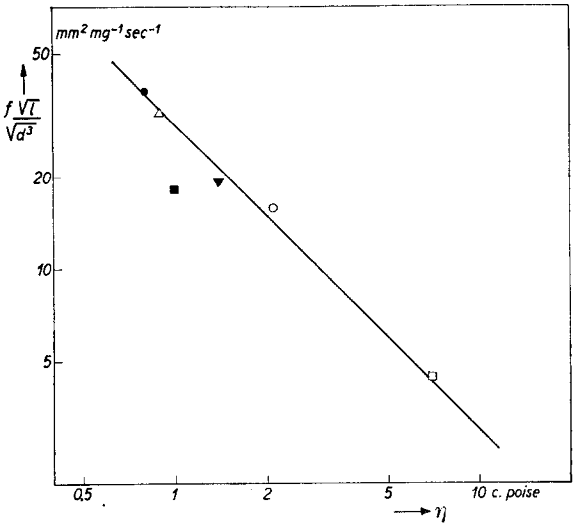


Fig. 5. Relation between $f\sqrt{l/d^3}$ f and η. For the various fluids the same symbols are used as in Fig. 3. All measurements are made at 20° C.

We ultimately arrive at a relation between u and ΔP:

$u = f\Delta P$ in which $f = 30/\eta\cdot\sqrt{d^3/l}$ approximately (2).

Speed of response of a respirometer

In many cases a respirometer is used to follow a constant exchange of gas. After starting a measurement by closing stopcocks or tipping in a reagent it will last a definite time before the index fluid moves with a constant rate, proportionally to the pressure change. The time until, *e.g.*, 90% of the ultimate rate is reached in many cases cannot be neglected.

When the gas exchange under measurement is not constant this time has to be known, and if possible adapted to the phenomena studied.

Diffusion rates in liquid and interphases usually play the most important rôle in this respect. But it will be shown here that even the time delays, inherently due to the mere physical dimensions of gas phase and index fluid column, often cannot be neglected.

If a constant gas evolution (B–ml/sec) starts at $t = 0$ in one of the vessels of a differential respirometer ($V_0 = V_m = V_c$), the displacement (x) of the index fluid at time t obeys the formula:

$$\frac{dx}{dt} = \frac{1}{\tau}(u_0 t - x) \tag{3}$$

in which u_0 represents the ultimate velocity: $B/2c$, *cf.* (1).

The time of response can be characterized by τ:

$$\tau = \frac{V_0}{2cP_0f} \tag{4}$$

in which P_0 is the initial pressure in the vessels, *e.g.* barometric pressure, and f can be estimated according to (2). The solution of (3) is (with $x = 0$ at $t = 0$):

$$= u_0 t - u_0 \tau \left\{1 - \exp.\left(-\frac{t}{\tau}\right)\right\} \tag{5}$$

$$\text{and } \frac{dx}{dt} = u_0\left\{1 - \exp.\left(-\frac{t}{\tau}\right)\right\} \tag{6}$$

this is illustrated in Figs. 6 and 7.

In order to measure B, we have to wait until the velocity of the drop $\frac{dx}{dt}$ is sufficiently close to u_0. *E.g.* $\frac{dx}{dt} = 0.9\,u_0$ when $t = 2.3\,\tau$.

τ can be calculated with the aid of (2) and (4) or can be measured directly, *e.g.*, by giving an impulse of pressure in V_m.

The results of both ways of estimation agreed within 10%.

Fig. 8 shows the relation between τ and d, calculated for a volumeter, the dimensions of which are given in the legend.

Minimum effective pressure, necessary to induce movement

As was shown above, a drop of fluid in a capillary does not move freely and it can be imagined that by decreasing ΔP a limit is reached beyond which movement stops completely, or beyond which it is possible to sustain movement but not to induce it. If such an effect really exists, we have to include it in our considerations.

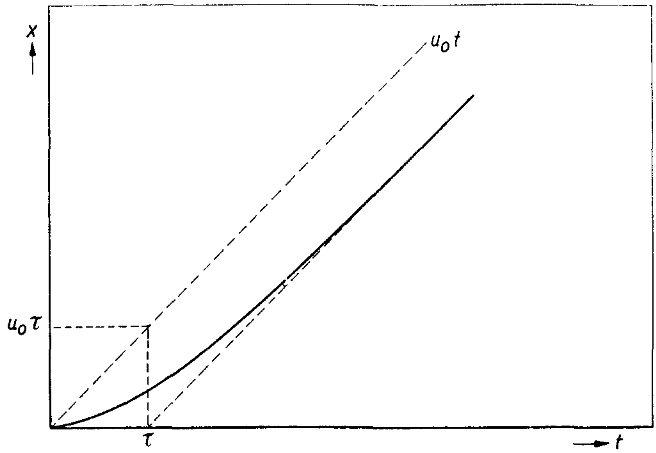


Fig. 6. See text.

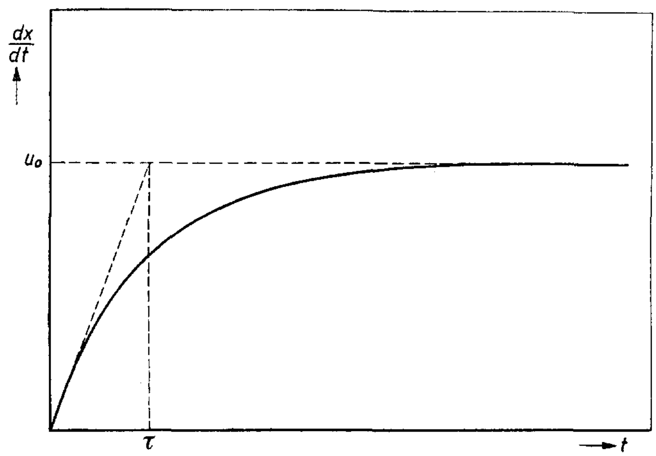


Fig. 7. See text.

The smallest effective pressure—ΔP_{min}—giving a just perceptible movement (in these experiments defined as 0.1 mm per 15 minutes) was estimated for various lengths of drop and capillary diameters in the following way:

A drop of fluid was forced through a capillary in order to obtain ideal wetting. After being kept horizontal the capillary was given a stepwise increasing inclination,

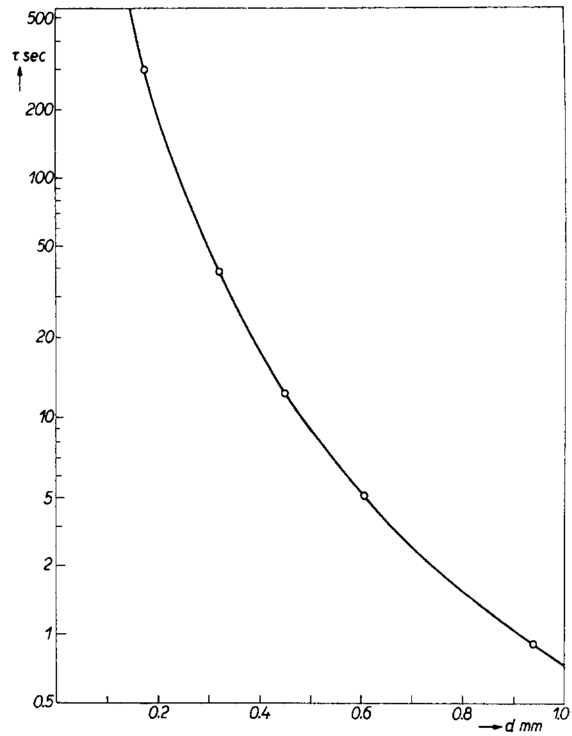


Fig. 8. Relation between τ and d in a volumeter: $V_m = V_c = 40$ ml, $P_0 = 10^4$ mm H_2O, $l = 7$ mm, index used: *Iso*valeric acid, 20° C.

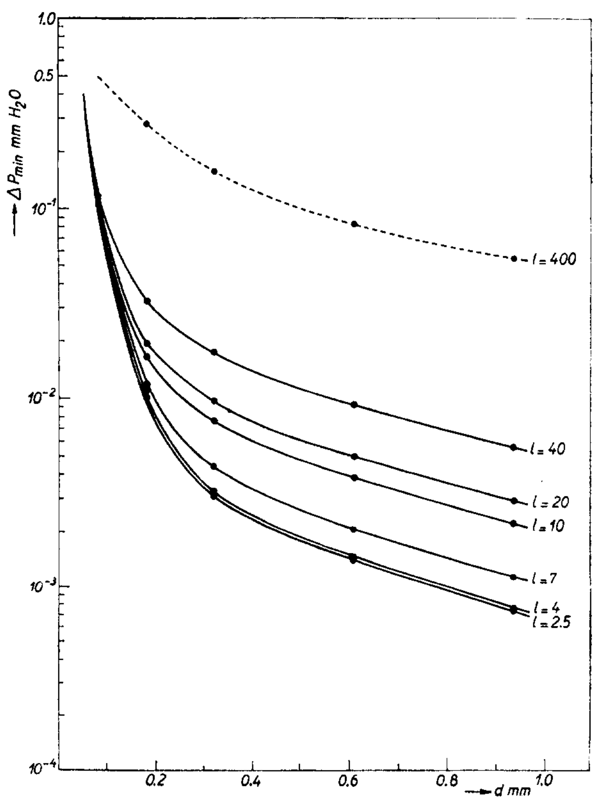


Fig. 9. Relation between ΔP_{min} and d for various lengths of drop (l in mm). *Iso*valeric acid, 20° C.

until movement was perceptible. The values found were mutually corrected in a graphical way. The results for *iso*valeric acid are given in Fig. 9. The curve for $l = 400$ mm was obtained by graphical extrapolation from the relation between ΔP_{min} and l. These data all are obtained with capillaries cleaned by draining with hot chromic acid, distilled water and alcohol.

In a respirometer, generally the conditions will be less ideal. In most cases the reaction chambers are saturated with water vapour, which after some time may lead to the occurrence of a water film on the capillary wall, and on first sight the use of a water drop seems to be indicated.

However, except in capillaries immediately used after rigorous cleaning, Brodie solution or water provided with various kinds and amounts of wetting agents proved to be unsatisfactory, giving high and unreproducible values for ΔP_{min}.

A hydrophobic fluid (xylol) was tested in a capillary slightly wetted with water. It appeared that in this case ΔP_{min} was about doubled, while f remained practically constant.

Best results were obtained with valeric acid showing good wetting properties even in inadequately cleaned capillaries, and in the presence of water. Moreover valeric acid fulfils the demand of having a vapour tension low enough to secure freedom from errors resulting from irregular evaporation.

In practical applications ΔP_{min} may differ to an unpredictable amount from the values given above. For instance it can be largely enhanced by grease films on the capillary wall, or largely reduced by mechanical vibrations induced by shaking of the apparatus. The values given above mainly serve as an indication of the order of magnitude and as a starting point for discussion.

We should point to the abnormal behaviour shown by mercury columns:

ΔP_{min} necessary to induce movement proved to be very large compared with ΔP_{min} to sustain movement.

The relatively small influence of l in this case indicates an influence of the high surface tension of mercury. These results (Fig. 10) indicate that mercury manometers with narrow legs have to be used with great caution.

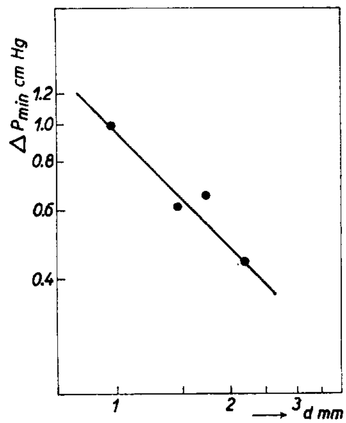


Fig. 10. High values of ΔP_{min} shown by mercury. In the range studied appr. $\Delta P_{min} = 1/d$

Influence of τ and of ΔP_{min}

In a respirometer the indicator can be used in two ways:

a. The displacement of the fluid column pro unit of time can be measured in order to compute the corresponding gas exchange. When a constant gas evolution of B ml/sec starts in a reaction chamber of a differential respirometer ($V_m = V_c = V_0$) at the time t_0, first a pressure ΔP_{min} has to be built up before movement of the indicator starts.

This takes a certain time: $t_h = \frac{V_h \Delta P_{min}}{B P_0}$. After this time the velocity of the indicator increases and approaches its ultimate value u_0 in the way expressed by (6) and (4).

Under these conditions the sensitivity itself of a respirometer is not influenced by the indicator, only a time-lag $\sim (t_h + 2.3\,\tau)$ has to be considered and the time of measurement adapted to it (see above). A great disadvantage of this type of volumeter in practical use is the small volume of its capillary, restricting the possibility of following large or long lasting gas exchanges. Moreover irregularities of the capillary influence the results.

These difficulties are overcome by:

b. The use of the fluid column as a zero indicator.

In this case we compensate the gas exchange in V_m by changing the volume of V_m or V_c in an accurately measured way.

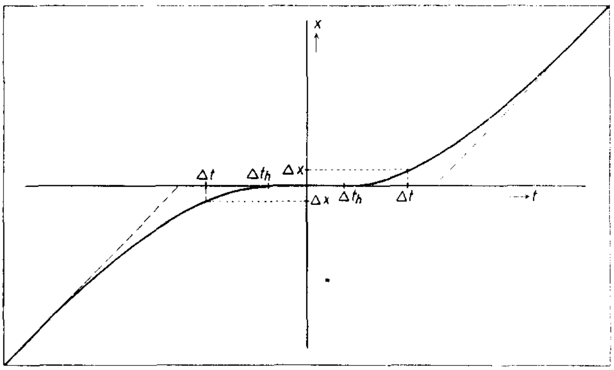


Fig. 11. See text.

The inaccuracy in the zero adjustment caused by τ and ΔP_{min} leads to an inaccuracy $\pm \Delta V$ in the estimation at the time t of the amount of gas evolved (Bt). An estimation of this error can be obtained in the following way. Suppose the drop has been put exactly in zero position and a constant evolution starts, then according to the foregoing it will last a definite time (Δt_h) before movement starts according to Fig. 11.

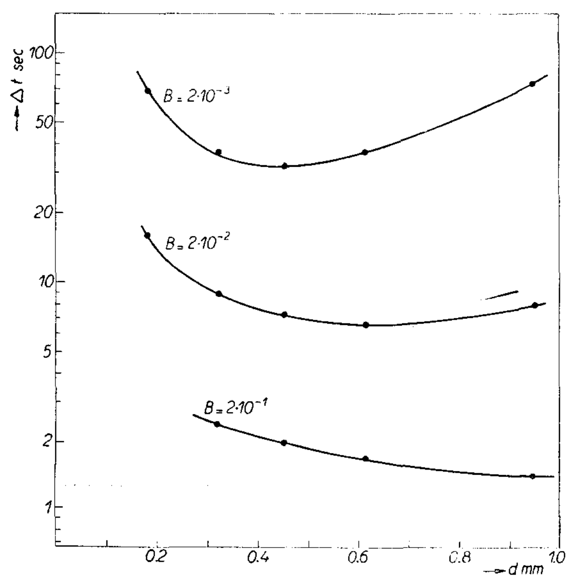


Fig. 12. Δt calculated for various capillary-diameters and rates of gas evolution (B in ml/sec).

When a displacement Δx of the drop just can be perceived we can repeat compensation earliest after a time Δt has elapsed. During this time an amount of gas $B\Delta t$ is evolved and this amount represents the inaccuracy in the readings: $\Delta V = B\Delta t$.

Acc. to (5) we can compute ($\Delta t - \Delta t_h$) by solving y (*e.g.* graphically) from:

$$z = y - 1 + e^{-y} \text{ in which } z = \frac{x\Delta}{u_0 \tau},$$

$$y = \frac{\Delta t - \Delta t_h}{\tau}.$$

For instance we calculated Δt for several values of d and B for the volumeter described in the legend of Fig. 8, the just perceptible displacement being 0.1 mm ($\Delta x = 0.1$ mm).

The results given in Fig. 12 show that an optimal value of d exists for which Δt is minimal. t_h proves to be a small part of Δt, so fluctuations in ΔP_{min} (*cf.* § 4) do not seriously affect Δt.

When choosing a volumeter for which $V_0 = V_m = V_c = 50$ µl, $l = 7$ mm, $B_{min} = 10^{-5}$ µl/sec and $\Delta x = 0.005$ mm we calculated for $d = 0.18$ mm: $\Delta t = 37$ sec; for $d = 0.32$ mm, $\Delta t = 82$ sec.

Measuring a gas evolution by adjusting and reading the compensator at fixed intervals during a time (t), each reading represents the amount of gas evolved plus or minus ΔV (*cf.* Fig. 13). The slope of the dotted curve in Fig. 13 representing B can be defined with an accuracy $\Delta B < \frac{2\,\Delta V}{t}$. Since $V = B \cdot \Delta t$: $\frac{\Delta B}{B} < \frac{2\,\Delta t}{t}$.

For example in the volumeter described earlier (Figs. 8 and 12) fitted with a capillary $d = 0.45$ mm, a constant evolution $B = 2 \cdot 10^{-3}$ µl/sec can be measured in $t = 10$ min with an accuracy of 10% when taking two readings at t_0 and $t_0 + t$, and more accurately when taking more readings during this interval. It is remarkable that for every set of conditions and (minimum) rate of gas exchange an optimal capillary diameter (which happens to fall within usual sizes) exists. A similar situation is found for the type of respirometers dealt with in a.

Both τ and ΔP_{min} do not restrict the sensitivity in sensu strictu.

The application of narrow capillaries is mainly restricted by the considerable timelags occurring.

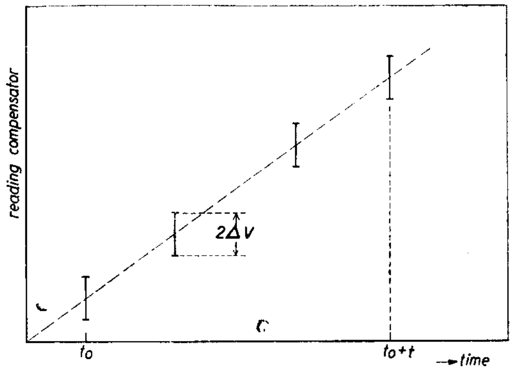


Fig. 13. See text.

Influence of temperature fluctuations

Any *change* of the *temperature difference* between V_m and V_c (Fig. 1) (this difference in most cases equals zero) will cause a pressure change, leading to a displacement of the index fluid.

In a volumeter a change ΔT of this difference during the time of measurement will cause an error ΔV in the estimation of the amount of gas V_e evolved:

$$\Delta V = \frac{V_m\,\Delta T}{T_{\text{abs}}}\,\frac{\Delta V}{V_c} = \frac{V_m\,\Delta T}{V_c\,T_{\text{abs}}}$$

In the first volumeter, described in the foregoing, the smallest amount of gas measurable in 10 minutes amounted to $2 \cdot 10^{-3}$ µl/sec = 1.2 µl/10 min. In order to keep errors due to temperature fluctuations below, *e.g.*, 5%, ΔV has to be smaller than 0.06 µl and $\Delta T < 5 \cdot 10^{-4}$ °C.

For the second volumeter we find $\Delta T < 2 \cdot 10^{-3}$ °C for $B_{min} = 10^{-5}$ µl/sec.

It appears therefore that the mere use of a differential apparatus does not secure absolute freedom from temperature fluctuations. Under certain conditions the sensitivity of the apparatus is mainly limited by the thermostat inconstancy.

SUMMARY

The movement of fluid columns in glass capillaries was studied in order to obtain more insight in the restrictions introduced by their use as an indicator in manometric and volumetric apparatuses. It appeared that these restrictions mainly concern the limitation of the speed of response. The influence of other factors is discussed.

RÉSUMÉ

Nous avons étudié le mouvement des colomnes de liquide dans des capillaires de verre, à fin d'examiner les réstrictions introduites par leur usance indicatrice dans des appareils manométriques et volumétriques. La réstriction principale apparaït d'être constituée par limitation de la vitesse de réaction.

Nous avons discuté l'influence d'autres variables.

ZUSAMMENFASSUNG

Die Bewegungen von Flüssigkeitsäulen in Glaskapillaren wurden untersucht, damit ein Einblick in den Beschränkungen, welche ihre Anwendung in manometrischen und volumetrischen Apparaten hervorruft, erhalten wurde. Es ergab sich, dass hauptsächlich die Einstellungsgeschwindigkeit limitiert wurde.

Der Einflusz von verschiedenen Faktoren wurde besprochen.

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