XLI. A METHOD OF MICROTITRATION.

By POUL BRANDT REHBERG.

From the Laboratory of Zoophysiology of the University of Copenhagen.

(*Received February 27th, 1925.*)

During a research to be published later it became necessary to obtain a continuous series of blood ammonia determinations on myself or other human subjects, but this object presented the serious difficulty that, according to the methods at present in use, about 10 cc. of blood would be required for each determination. I therefore attempted to devise a method in which only 1 cc. of blood should be required. One of the factors which make the determination of blood ammonia so difficult is that the quantity is extremely small.

The amount of ammonia in 100 cc. of blood is supposed to lie between 0·1 and 0·2 mg., which means that 1 cc. of blood can yield only 0·001 or 0·002 mg. NH_3 or about 0·1 mm.^{3} of a normal solution.

It would be quite impossible to titrate this amount by any ordinary method, but, on consideration, I found that it would not only be possible to titrate the ammonia from 1 cc. of blood, but that by a suitably arranged microtitration it could be done with far greater accuracy than the amount from 10 cc. is titrated by a macrotitration.

As I think that the method of microtitration arrived at will make all titrations of small quantities much more accurate than is possible with ordinary macrotitrations, the method and the theory upon which it is based are published separately here.

The object of a titration is to measure how much of a solution of known concentration one must add to a certain volume of another solution of unknown concentration in order to bring a certain reaction between the two fluids to completion.

This involves two subjective estimations by the person who titrates: first to judge that the reaction is completed, secondly to measure the fluid added. That the reaction is completed is judged by means of an indicator[1]; the measuring is done by means of a burette.

The errors involved in a titration are, as in other cases, partly accidental, partly systematic. The accidental errors are mainly two: first, a misjudging of the colour of the indicator, so that the p_H wanted as end point is not reached or is passed—usually one may expect the actual end point to lie within 0·3 p_H-unit on each side of the p_H wanted. The second accidental error is due

[1] In the following I shall speak only of acidimetric and alkalimetric titrations, though the reasoning will hold also for the other forms. For a complete discussion of the theory of these titrations see Bjerrum [1914].

to the reading of the burette and depends mainly on the construction of this piece of apparatus.

The systematic errors depend on the fact that free acid and alkali are still present in the solution when the end point is reached. The acid and alkali are present partly as ions and partly in undissociated form. The magnitude of the errors depends on the p_H of the end point, that is on the indicator used, on the volume of fluid at the end of the titration, and on the dissociation constants of the acid and alkali involved.

If, for instance, we titrate 10 cc. of $N/10$ acid, diluted to 90 cc., with $N/10$ alkali, and as end point use the point where methyl orange just begins to change colour (p_H 3)—we shall have at the end point 99 cc. of a p_H of 3 or 99 cc. $N/1000$ acid, corresponding to approximately 0·1 cc. N acid. If we titrate to p_H 4 the error will be only 0·01 cc. of N acid.

If we titrate the 10 cc. of $N/10$ acid directly with $N/10$ alkali to p_H 3, the error will be 0·02 cc. N acid.

For this error due to the H ions present in the solution at the end point of the titration we may give the following formula:

$$H^{\cdot}\text{-error} = C_{H^+} \times v \text{ cc. } N \text{ solution}$$

where C_{H^+} is the $H^{\cdot}$-concentration and v the volume at the end of the titration.

For the OH-ion-error we have the analogous formula:

$$OH'\text{-error} = C_{OH^-} \times v \text{ cc. } N \text{ solution}$$

where C_{OH^-} is the OH′-concentration at the end of the titration.

These two errors therefore depend on the p_H of the end point and on the volume. To keep both of them as small as possible one would have to titrate to p_H 7.

If we titrate to a p_H on the acid side of p_H 7, then the OH-ion-error is small and the H-ion-error large, and if the end point is alkaline then the OH-ion-error will become large though the H-ion-error is small. If these were the only errors with which to reckon the best procedure would evidently be to titrate to p_H 7 where both errors are small. But this is often impossible because what we titrate is only the dissociated alkali and acid, and if at the end point we have undissociated acid or alkali left an error will arise. How much undissociated acid and alkali we have at a certain p_H depends on the dissociation constants of the substances in question.

Bjerrum gives for this error the following formula:

$$\text{relative acid-error} = 10^{p_{acid}-p_T} \times \text{the titrated amount of acid,}$$

where $p_{acid} = -\log K_{acid}$; K_{acid} being the dissociation constant of the acid and p_T the p_H of the end point.

The formula for the error due to undissociated alkali is:

$$\text{relative alkali-error} = 10^{p_{alk}-(14-p_T)} \times \text{the amount of titrated alkali,}$$

where $p_{alk} = -\log K_{alk}$; K_{alk} being the dissociation constant of the alkali.

The magnitude of these errors therefore depends on the dissociation-constant of the acid and alkali and of the p_H of the end point.

If one wants the error not to exceed 0·1 %, one must select the p_H of the end point so that it is 3 p_H-units to the alkaline side of p_{acid}, as defined above, or 3 p_H-units to the acid side of p_{alk}.

If for instance one wants to titrate acetic acid and keep this error below 0·1 % then, because the dissociation constant of acetic acid is $10^{-4.75}$, p_{acid} is 4·75, and we must titrate to an end point of p_H 4·75 + 3 = p_H 7·75. At this point the H˙-error and the OH′-error are also small, and we are therefore able to titrate acetic acid accurately with an indicator which gives an end point of p_H 7·75 or more alkaline as long as the end point does not differ so much from p_H 7 that the OH′-error is appreciable.

But if we want to titrate H_2S ($K_{H_2S} = 10^{-7}$) the p_{acid} of which is 7·00, we shall, in order to keep the relative acid-error below 0·1 %, titrate to p_H 10. For p_H 10 we get an OH′-error of $10^{-4} \times v$ cc. N solution. If the volume is 100 cc. the error is $10^{-4} \times 100 = 0{\cdot}01$ cc. N solution, and to make this error 0·1 % we must have at least 10 cc. N solution in the 100 cc. If the amount of H_2S is less than that, the titration can be performed with an accuracy of 0·1 % only if the volume is reduced.

Generally one may say that if the dissociation constant of either the acid or the alkali involved in a titration forces one to titrate at a p_H higher or lower than 7 in order to keep the relative acid-error low, then the only possible way of keeping down the OH′ and the H˙-errors is to reduce the volume; small amounts of acid or alkali cannot, therefore, be titrated exactly when using diluted solutions of low normality.

If for instance we try to titrate 10 mm.³ N acid with N/100 alkali and methyl red as indicator (end point p_H 5) in 100 cc., 10 cc. and 1 cc., then we shall get H˙-errors which are

$10^{-5} \times 101 = 0{\cdot}001$ cc. $= 1$ mm.³ N acid = 10 %.
$10^{-5} \times 11 = 0{\cdot}00011$ cc. $= 0{\cdot}11$ mm.³ N acid = 1·1 %.
$10^{-5} \times 2 = 0{\cdot}00002$ cc. $= 0{\cdot}02$ mm.³ N acid = 0·2 %.

But if we want to keep the end volume small, the amount added during the titration must be small also, and as it is important to keep the end volume nearly constant in spite of variations in the amount of fluid added during the titration, this amount must be small in comparison with the end volume. If for instance we want to titrate in 1 cc., then the amount of titration fluid must be kept below 0·1 cc. This would involve the use of a stronger titration fluid, which in itself is an advantage as in most cases it is more exact and may be kept longer. But we must then be able to add this fluid in amounts smaller than 0·1 cc. with an accuracy of fractions of 1 %. With an ordinary burette this is quite impossible, and even with Bang's microburette the accidental error of reading is easily 0·01 cc. or 10 % of what we want to add.

If we want to titrate in this way we must evidently use a very narrow burette so that 0·1 cc. can be suitably divided and read to say 0·1 mm.³. This is easily done, but it is obviously impossible to add fluid from such a burette in amounts as small as 0·1 mm.³ in the ordinary way by means

of a stopcock. Krogh [1908], in his apparatus for micro gas analysis, employed an iron screw working in mercury to move the fluid in the capillary tube of the apparatus. Using this principle it is easy to drive out from a capillary tube as small an amount of fluid as desired, but the fluid will gather at the

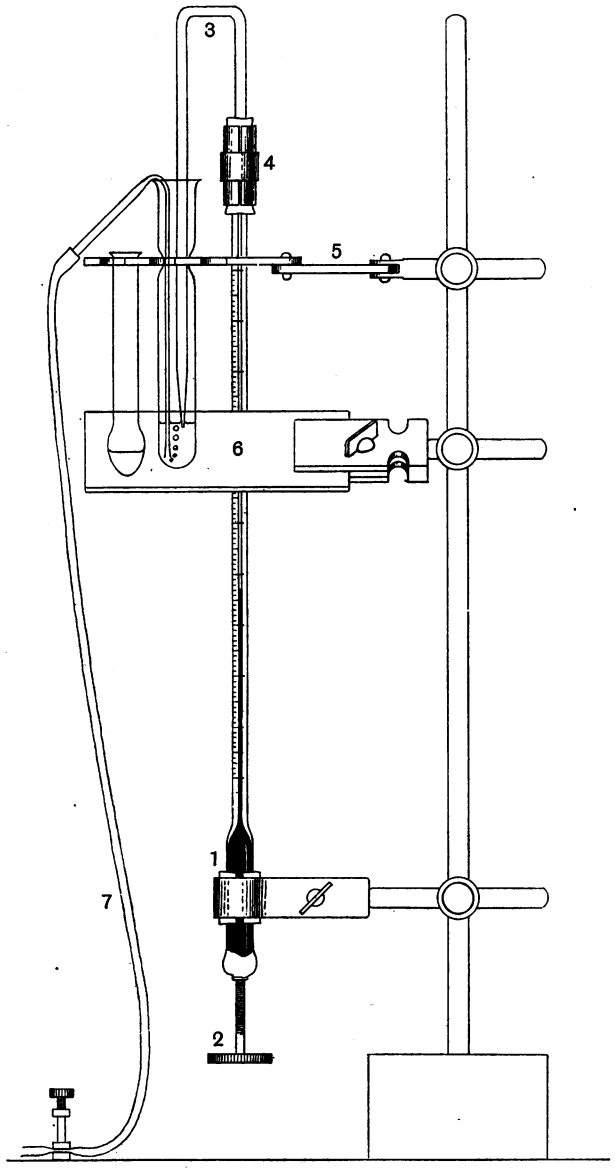

Fig. 1.

tip as a drop, and it will therefore be necessary during the titration to dip the tip of the burette into the fluid which is being titrated. As the outcome of such reasoning the apparatus to be described has been constructed.

The burette[1] consists of a capillary tube about 6–8 mm. thick with a bore of about 0·8–0·9 mm., so that the length of 0·1 cc. is between 15 and 20 cm. It is divided in mm.[3] and calibrated by measuring the length of a weighed drop of mercury at different positions in the tube. At the lower end the tube is widened (1) to take the screw (2), which is of steel and sealed in with picein or sealing wax. This part of the burette is filled with mercury, and by turning the screw the whole of the burette may be filled with mercury. The dimensions of the screw are such that one revolution corresponds to about 4 mm.[3].

As the mercury has to be below the water-solution the burette is mounted as shown in the figure. The tip is then most conveniently made as a loose part (3) and connected with the burette itself by means of a piece of rubber tubing, the connection being strengthened by means of a brass tube cut in two halves and kept together by means of a ring (4). The tip is made of a capillary tube with a bore of 0·8–1·0 mm. and holds more fluid than is used during a titration.

The burette is mounted on a stand which also carries a movable arm (5) arranged to hold the test-tubes used in the titration (two different types are shown in the figure) and a plate of white glass (6) used as background during the titration.

The arm takes three test-tubes, one of which is filled with the fluid used in the titration, one with the indicator used in a buffer solution of the p_H wanted as end point, and the third is the tube in which the titration is carried out.

If the solutions used are weaker than $N/10$ the test-tubes used should be made of a resistant glass (Jena or Pyrex).

To fill the burette for the first time with a fresh fluid the tip is removed, the mercury brought up to the top and the burette filled from a pipette by lowering the mercury, and then washed out once or twice. The rubber tube is put on the tip and this is then filled and connected with the burette, care being taken to avoid air bubbles (a single air bubble of 1–5 mm.[3] travelling forward and backward with the movement of the fluid does not interfere with the accuracy of the titration).

When the burette has been used for a titration it can be refilled by dipping the tip in the fluid and lowering the mercury.

The burette is used in the following way: on filling, the meniscus of the mercury is brought a few mm. below the zero point, the tube containing the titration fluid is removed and the tip carefully wiped with a piece of filter paper to remove all fluid adhering to the outside. Just before the titration the mercury meniscus is brought to zero and the small drop now adhering to the tip cautiously removed by touching with filter paper. All readings are made by means of a lens mounted in a holder[2], which is laid against the burette

[1] The burette with stand, as in figure, may be obtained from the workshop of the zoophysiological laboratory, Ny Vestergade 11, Copenhagen, at a price of 50 Danish kroner.

[2] As used in the micro-analysis of gases by Krogh.

in order to minimise the error due to parallax. In this way the error of the reading may be kept in the neighbourhood of 0·1 mm.³. By means of a pipette a suitable amount (0·5–5 cc.) of the fluid to be titrated is measured out in a test-tube and the indicator added.

The amount of the indicator used must, in all titrations which are to be compared, remain the same in order to keep constant the error due to participation of the indicator in the reaction. This error is under these circumstances greater than in an ordinary titration, because the amount of indicator needed to give a sharp end point in such a small volume is comparatively large. For this reason the indicator is best added as 0·1 cc. of a very dilute solution, or a suitable amount of indicator is once for all added to the stock solution.

The tube is now placed in the slot of the arm and raised until the tip of the burette just dips into the fluid. During the titration the fluid is mixed by means of a stream of small air bubbles from a very fine glass tube dipping into the fluid and connected with air pressure (7). The air is filtered through a tube filled with soda-lime to remove carbon dioxide. If air pressure is not available the small amount of air needed may easily be supplied from two 5-litre bottles half filled with weak NaOH-solution and connected to form a gasometer. The bubbling is regulated by means of a clip. By turning the screw of the burette the titration fluid is now added in small amounts till the end point is reached, and the burette is then read again.

To show the accuracy of the method I append a few examples.

By means of a microburette from 5 to 100 mm.³ $N/200$ H_2SO_4 were measured into titration tubes. 1 cc. H_2O and 0·1 cc. of a 0·001 % solution of methyl red were added and the tubes titrated with $N/200$ NaOH. The volume in these titrations was at the end between 1·1 and 1·3 cc. If we assume that we are able to hit the p_H wanted (5·5) within 0·5 p_H, we shall at the end of the titration have a p_H varying between p_H 5·25 and p_H 5·75. These two values give the following results for the $H^{\cdot}$-error:

$10^{-5\cdot25} \times 1\cdot2$ cc. N $H_2SO_4 = 0\cdot0067$ mm.³ N $H_2SO_4 = 1\cdot35$ mm.³ $N/200$ H_2SO_4

and

$10^{-5\cdot75} \times 1\cdot2$ cc. N $H_2SO_4 = 0\cdot0021$ mm.³ N $H_2SO_4 = 0\cdot4$ mm.³ $N/200$ H_2SO_4.

The accidental error in judging the end point will therefore give rise to variations in the result of $\pm$ 0·5 mm.³.

Moreover, we have in each of these titrations four different readings of the burettes on each of which there may be an error of 0·1 mm.³, so that we must expect a maximum error of $\pm$ 0·9 mm.³. The mean error will of course be smaller.

The following table shows that two titrations of the same quantity of $N/200$ H_2SO_4 may differ from 0 to 1·3 mm.³.

The table shows, as might be expected, that the results have a systematic error; the quantity of NaOH used is less than the theoretical. This is partly due to the fact that the alkali is not exactly $N/200$ and partly to the $H^{\cdot}$-error

mm.³ $N/200$ acid measured	mm.³ $N/200$ NaOH used in titrations		Difference between the two titrations	mm.³ NaOH + 1·55 / 0·954	
5	2·5	3·5	1·0	4·05	5·05
10	8·0	8·5	0·5	10·00	10·50
20	17·0	17·4	0·4	19·40	19·90
30	26·8	26·9	0·1	29·75	29·85
40	35·9	36·8	0·9	39·30	40·15
50	46·2	46·9	0·7	50·00	50·75
60	56·0	56·1	0·1	60·40	60·50
70	65·5	65·7	0·2	70·35	70·50
80	73·9	75·2	1·3	79·00	80·50
90	84·5	85·1	0·6	90·20	90·80
100	93·1	94·3	1·2	99·20	100·40

and the participation of the indicator in the reaction. If we take these errors into consideration we get the following formula:

$$\text{mm.}^3 \text{ acid} = \frac{\text{mm.}^3 \text{ NaOH} + y}{x}$$

and from the results in the table it is possible to find the values of x and y by the method of least squares.

They are:

$$x = 0{\cdot}954 \text{ and } y = 1{\cdot}55,$$

which means that 100 mm.³ acid is equal to 95·4 mm.³ NaOH and that the results obtained by the titrations are moreover 1·55 mm.³ too low on account of the indicator and H˙-errors.

In the last two columns of the table are given the values of the titrations corrected in this way. The mean error in a single example of these corrected titrations is 0·52 mm.³ $N/200$ solution, which corresponds, if used for instance in an ammonia determination, to 0·000036 mg. N.

In daily use it is of course inconvenient to find the titre of the alkali with respect to the acid by means of least squares. It is best ascertained by an ordinary macrotitration. With stronger solutions the error is negligible, so that the microtitration is sufficient.

If we try to titrate to a point closer to p_H 7 we shall, as has been said, have a smaller H˙-error. In a series of titrations of $N/200$ acid in a volume of 1 cc. to p_H 6·25 using bromocresol purple as indicator I found a mean error in a single determination of 0·27 mm.³ $N/200$ H_2SO_4 or 0·000019 mg. ammonia N.

Using stronger solutions still better results are obtained: working with methyl red and 0·1 N solutions, and titrating in 1 cc., I found a mean error of 0·2 mm.³ and in 5 cc. of 0·3 mm.³.

This accuracy is sufficient for most purposes, but if still greater accuracy is wanted it will be quite possible to get a little further. The burette may easily be made to measure out still smaller fractions of 1 mm.³—the difficulty will be to observe the change in colour in the small volume required in such cases, but I think that it will be possible to titrate in a volume of about 0·1 cc. which would increase the accuracy to a considerable extent.

As examples of the practical use of the apparatus some methods for the determination of several blood constituents will be published shortly.

SUMMARY.

1. In a brief discussion of the theory of titration the influence of total volume is considered, and it is shown that many titrations can be performed with advantage as microtitrations.

2. An apparatus for microtitration and its use is described. The apparatus allows the addition of fluid from a burette in accurately measured quantities down to 0·1 mm.^{3}.

I wish to express my sincere gratitude to Prof. Krogh for his kind interest and helpful advice during the work.

REFERENCES.

Bjerrum (1914). Die Theorie der alkalimetrischen und azidimetrischen Titrierungen. Stuttgart.
Krogh (1908). *Skand. Arch. Physiol.* **20**, 279; or in Abderhalden, Biochem. Arbeitsmethoden **8**, 1918.