THE FALLING DROP METHOD FOR DETERMINING SPECIFIC GRAVITY.*

BY HENRY G. BARBOUR AND WILLIAM F. HAMILTON.

(From the Department of Physiology and Pharmacology, School of Medicine, University of Louisville, Louisville.)

(Received for publication, June 14, 1926.)

Recognizing the increasing importance attached to water exchange in the body for the study of many clinical and physiological conditions we (1) described in 1924 a new and convenient method of determining specific gravity. Obvious advantages attach to densimetry performed with 1 drop of body fluid, in less than a minute's time. While these ends were attained by the method as first described it has now been so modified by simplification, refinement of sensitivity, and extension of scope, as to demand an entirely new and more complete description.

General Principles.

The principle of the method involves timing the fall of a drop of body fluid of known size, through a definite distance in a mixture non-miscible with the fluid. This mixture should have a low viscosity and a specific gravity somewhat below that of the fluid to be tested. It consists of two substances, one heavier and one lighter than the range of fluids to be tested, so that by adjusting the proportions, the specific gravity of the mixture can be adapted to the expected conditions. Two satisfactory substances of sufficiently low volatility and viscosity were found in xylene and bromobenzene.

A heavier drop will fall faster than a drop nearer the density of the XBB mixture, and our previously published curve exhibits the values for density differences between drop and mixture in terms of falling time. The relative accuracy of these values is

* The development of the method herein described has been assisted by a grant from the Ella Sachs Plotz Foundation.
Specific Gravity Determination

extremely great; the absolute accuracy, however, in the original method hinged upon the absolute specific gravity and viscosity of the XBB mixture. Both of these factors are influenced by the temperature and degree of purity of the organic fluids. The effect of temperature upon XBB density was formerly followed with a Westphal balance; its effect upon viscosity is so small that it did not at first attract our attention.

By eliminating the Westphal balance and similar cumbersome complements of hydrometry as well as by diminishing tube length and bore, the apparatus is rendered portable. Sampling is facilitated by reducing the required drop size to 10 cmm. The present modification not only obviates errors due to temperature differences in various parts of the tube, but also eliminates the XBB viscosity changes due to temperature.

The most essential improvement in the procedure is that no determination of the exact density of the XBB mixture is required but merely the falling time of a salt solution of standard density. This serves as an index to the significant relations between drop and XBB mixture obtaining at the moment.

Procedure.

A standard solution of potassium sulfate of known density (Table I) is the basis for the determinations. The proper XBB mixture is held in a tube of standard bore. Just below the surface of the XBB a drop containing exactly 10 cmm. of standard solution is released from the pipette to be described. The falling time, over a distance of 30 cm., is determined by a stop-watch. Applying to the alignment chart a ruler or taut thread against the proper falling time and room temperature, one now reads the apparent density difference between the standard and the XBB.

The fluid of unknown density is at once tested in the same way and its value read.

It is now only necessary to subtract algebraically the standard XBB apparent density difference from the unknown XBB apparent density difference, and add the result to the standard K_2SO_4 solution density. This gives the specific gravity of the tested body fluid.

Standard Solutions.—Potassium sulfate provides satisfactory solutions of standard densities because of its stability and the
fact that it is not hygroscopic. It dissolves so slowly that in preparing the solutions it is convenient to boil them. The standard solutions (which include distilled water) necessary for the whole range of body fluids are given in Table I. They are made up at 20°C. with freshly distilled water and keep indefinitely in siphon bottles under oil. The ratio of concentration to density is sufficiently constant to permit, by simple proportionate mixing of two standard solutions, the attainment of any intermediate density desired.

**Pipette.**—The pipette is easily made from a piece of uncon-
Specific Gravity Determination

stricted capillary tubing, calibrated to deliver between marks 10 cmm. of clean mercury. The tubing of which the pipette is constructed must be of fine enough bore to give an interval of at least 4 cm. between marks. The lower graduation must be at least 3 cm. from the delivery end, which is ground to a conical point in such a way as to leave a very narrow rim.

It is convenient to use a rather long suction tube with mouth-piece. Since blood tends to clot on the walls of the pipette, it is necessary to wash with water, alcohol, and ether, between determinations. The first rinsing should be done while the drop is falling.

Delivery.—A drop of blood is handled as follows: The puncture is made in such a way that the blood flows freely, or a drop may be secured from a freshly drawn sample, which has undergone neither evaporation, coagulation, nor settling. The blood or other fluid is drawn into the pipette just beyond the upper mark, taking care that no air is sucked in. Should any air become included, the results will be valueless. The pipette is then held in a horizontal position and its outer surfaces wiped clean on filter paper. It is then tipped enough to return the lower end of the column of blood to the orifice of the pipette. The upper end of the column is adjusted to the upper mark, by gently stroking the tip with the finger. Applying gentle suction to prevent absolutely wetting the outside of the pipette, the end of the pipette is inserted just below the surface of the XBB in the tube. The suction is released and the top of the column allowed to fall to the lower mark. With the top of the column exactly on the lower mark, the tip of the pipette is gently lifted through the surface film of the XBB mixture, releasing the drop.

Tube.—The tube to contain the XBB mixture should be about 50 cm. long, and must be so selected as to have a bore between 7.45 and 7.55 mm. This bore must be checked at both ends, one of which is then sealed. The measurement is conveniently made by inserting a tapered piece of sheet metal. Experiments with varying bore size have shown that a deviation from the 7.50 mm. bore amounting to 0.05 mm. introduces a change in the falling time corresponding to $2 \times 10^{-4}$ specific gravity for 18 seconds of time. Pipettes and tubes corresponding to our specifications can for the present be obtained at reasonable cost from Mr. J. H. Warner of this laboratory.
drops and $7 \times 10^{-5}$ for 40 second drops. Such an error would of course be automatically cancelled if blood and standard had the same falling time, but would increase as they differed in speed. These facts emphasize the importance of adhering rigidly to the bore specification.

At equal distances from the two ends of the tube, are the marks by which the fall of the drop is timed. They extend all the way around the tube and are exactly 30 cm. apart. Care must be taken to keep the tube perpendicular.

**TABLE II.**  
*Xylene-Bromobenzene Proportions Suitable for the Various Standards.*

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1.000</td>
<td>Cerebrospinal fluid, dilute urine, secretions, and exudates.</td>
<td>0.993</td>
<td>80.0</td>
</tr>
<tr>
<td>1.015</td>
<td>Plasma, serum, transudates, heavy urine, extremely anemic blood, etc.</td>
<td>1.003</td>
<td>78.5</td>
</tr>
<tr>
<td>1.035</td>
<td>Anemic blood and heavy secretions.</td>
<td>1.023</td>
<td>75.3</td>
</tr>
<tr>
<td>1.055</td>
<td>Normal and anhydremic blood.</td>
<td>1.043</td>
<td>72.1</td>
</tr>
</tbody>
</table>

*Xylene-Bromobenzene Mixture.*—Experience has drawn our attention to the viscosity of the mixture in which the drops are tested. We have been dissatisfied with the results obtained with mixtures containing different samples of commercial xylene. A mixture of high quality xylene and bromobenzene, however, has been found just as useful after long standing and marked evaporation, as when freshly prepared. Inexpensive Eastman Kodak Company products found satisfactory include two xylenes, No. T275 (technical $m$-xylene) and No. P460 (histological xylene), as well as bromobenzene No. 43.

A single XBB tube suffices in all ordinary experimental work because changes in specific gravity of more than 0.01 are unusual.
Specific Gravity Determination

Employing Table II which covers the whole density range of body fluids, the XBB mixture best adapted to test any given fluid can be readily selected and made up for immediate use.

In doubtful cases the XBB chosen should be the heaviest of the suggested mixtures in which the fluid to be tested is found to fall rather than float. Of course the standard K₂SO₄ solution selected is the lightest that will fall in the XBB mixture used.

The XBB mixture varies in density so much with temperature that a change of 1°C effects a change of 8 × 10⁻⁴ specific gravity. This means for example that at approximately 32°C, each XBB mixture in the table would be replaced by the one just above. Furthermore, evaporation produces a gradual change in density. It is easier to get accurate results if the specific gravity of the XBB is so adjusted as to make the drops fall slowly. The mixture may be changed to the proper density by adding a few drops of either xylene or bromobenzene and mixing thoroughly.

Before dropping blood it is well to drop a little saturated solution of a soluble salt (e.g., MgSO₄) to retain at the bottom of the tube any separating serum.

Examples of the Calculation.—To illustrate the use of the alignment chart let us assume that at a room temperature of 20°C, a standard drop of density 1.055 required 62 seconds to fall while a blood drop tested just afterwards required 48 seconds.

<table>
<thead>
<tr>
<th>Blood drop:</th>
<th>Standard drop: 1.0550:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Falling time = 48 sec.</td>
<td>Falling time = 62 sec.</td>
</tr>
<tr>
<td>Apparent density difference:</td>
<td>Apparent density difference:</td>
</tr>
<tr>
<td>0.0040</td>
<td>0.0028</td>
</tr>
</tbody>
</table>

True density difference between standard and blood: +0.0012
Density of blood: 1.0562

Or if the blood happened to be lighter than the standard, the density difference would, of course, be subtracted.

<table>
<thead>
<tr>
<th>Blood drop:</th>
<th>Standard drop: 1.0550:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Falling time = 62 sec.</td>
<td>Falling time = 23 sec.</td>
</tr>
<tr>
<td>Apparent density difference:</td>
<td>Apparent density difference:</td>
</tr>
<tr>
<td>0.0028</td>
<td>0.0113</td>
</tr>
</tbody>
</table>

True density difference between standard and blood: -0.0085
Density of blood: 1.0465
Fig. 1. Alignment chart for calculating specific gravity. A straight line connecting the observed falling time and room temperature readings intersects the density scale at the desired point.
Specific Gravity Determination

Experimental Basis of Alignment Chart.

The alignment chart (Fig. 1) is based upon the relations observed between falling time and apparent density difference at five different temperatures. Plotted on a squared background, these data give curves similar to Fig. 1 in our previous paper (1). They are the result of very careful pycnometric determinations of the densities of the XBB and of whatever standard solutions were dropped. The pycnometer used was a Sprengel tube of about 21.6 cc. capacity. To complete the measurements the coefficients of expansion of Sprengel tube, XBB, and salt solutions were all determined. It was thus possible to correct for slight deviations from the five standard temperatures. The accuracy of the pycnometry is within \( \pm 2 \times 10^{-5} \). The temperature was read to 0.01°C.

In casting the method in its final form many tedious hours have been saved by the use of the Foulk chain hydrometer (2). Professor Foulk of Ohio State University kindly prepared and sent us several forms of this ingenious and extremely sensitive instrument.

Once calibrated by appropriate pycnometry, the chain hydrometer affords a quick and accurate substitute for the Sprengel tube when sufficient fluid is available.

In measuring the falling times, the temperature was held nearly constant by keeping the XBB tube immersed in a large glass water bath. The water was kept stirred with air. Usually conditions were favored by the use of a constant temperature room. The bath temperature was frequently read and the density values corrected for every \( \pm 0.01^\circ \) temperature change.

The procedure has been carried out a great many times, always yielding curves similar to the original 1924 curve. Plotted on logarithmic paper these curves are found to be nearly linear; it is further found that each curve can be straightened by making at every point the same slight \( \pm \) correction. Each curve can, therefore, be represented by a simple logarithmic equation; but since the mathematical relationships differ with the temperature, it has not been thought necessary to present them in detail in connection with the use of the method.

Of course the change necessary to straighten each line is always cancelled out in comparing blood with standard, so that in using
Fig. 2. Relations between falling time and apparent density difference at various temperatures shown on logarithmic background. Each dot represents the mean of a series of four to nine determinations.
any of these straight lines, one is in reality referring to the empirically determined curve. It thus becomes evident that even for the purpose of constructing the alignment chart the exact density of the XBB mixture need never be determined.

A group of straight lines thus obtained at five different temperatures is represented in Fig. 2. Each point represents a series of 4 or more drops. Using D'Ocagne's method (concisely described by L. J. Henderson (3)) these lines were reduced to the alignment chart.

*Comparative Accuracy of the Method.*

We may here make a brief comparison of the falling drop specific gravity method with other procedures for determining the water content of body fluids. As will be shown below, the falling drop method is sensitive to $1 \times 10^{-4}$. This greatly exceeds in accuracy any hitherto described method for determining the specific gravity of minute quantities of fluid.

The most direct way of determining the water content of body fluids is, of course, by weighing the total solids. A large experience in this laboratory with the total solids of blood and of plasma or serum, has shown that with special care, they can be determined to within $\pm 0.30$ per cent of the solid content.

Hemoglobin and red blood cell determinations afford important indirect measurements of the water content of the blood. Leake (4) estimates the average error of the former method at $\pm 5$ per cent, of the latter at $\pm 2$ per cent with the usual clinical methods. It has certainly never been shown that either of them can be determined with a smaller error than $\pm 1$ per cent. The same may be said of the laborious (total N) methods for determination of plasma proteins.

The simple and comparatively accurate method of refractometry, which comes closer to determining water content than any other procedure except finding solids or specific gravity, avoids volumetric errors in sampling and yields with the Abbé instrument errors of the order of $\pm 1.2$ per cent of the plasma solids.

As regards the falling drop method, while volumetric errors in the sample are involved, it must be emphasized that they do not proportionately affect the result. For, due to the surface-volume
relations of the falling drop, any error tending to hasten the fall by increasing the volume is counteracted in part by a surface increase tending toward retardation.

To compare our specific gravity unit with the units of other methods for water determination in blood or serum, a list of approximate equivalents may be given:

<table>
<thead>
<tr>
<th>Property</th>
<th>Equivalent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific gravity</td>
<td>0.0001</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>0.2 per cent.</td>
</tr>
<tr>
<td>Red blood cells</td>
<td>10,000 per cmm.</td>
</tr>
<tr>
<td>Blood solids</td>
<td>0.04 per cent of whole blood.</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.08 per cent of plasma.</td>
</tr>
<tr>
<td>&quot; proteins</td>
<td>30 mg. per 100 cc.</td>
</tr>
<tr>
<td>&quot; refractive index</td>
<td>0.00005</td>
</tr>
</tbody>
</table>

Quantitative Estimation of the Error of the Method.

If our specifications are met, the only sources of error requiring further consideration concern making the standard solutions, measuring and timing the drop, and changes in temperature between the moments of dropping the standard and unknown solutions.

Errors in Density of Standard Solution.—A simple calculation will show that if the weighing is accurate to 133 mg. per liter, the density of the heaviest standard solution will be accurate to $1 \times 10^{-4}$. Since the solution is rather dilute the same will apply if the volumetric measurement is accurate to 2 cc. per liter. In making up the still more dilute standard for serum, etc., a proportionately greater volumetric error falls within the above mentioned density limit. Errors of this order will, of course, not appear in a solution prepared with ordinary care.

The error in measuring and timing the drop is exemplified in Fig. 3. Each point represents the probable error of a single drop calculated from a series whose average falling time is represented on the abscissa. The probable error, in seconds, of a single observation was first calculated from the variations of the series according to the conventional formula, $\sqrt{\frac{\Sigma V^2}{n}} \times 0.6745$, where $\Sigma V^2$ = the sum of the squared variations from the mean of the series and $n$ their number. The probable error thus calculated was translated into specific gravity by the use of the alignment chart, and this specific gravity error plotted as ordinate. The
Specific Gravity Determination

continuous curve represents the probable error in timing the drop introduced by the fact that the stop-watch unit is 0.1 second; each point, therefore, gives the density equivalent of 0.034 seconds for the drop speed indicated. As seen on the curve, which is logarithmic, this time error means more in specific gravity when the drops are falling more rapidly. Thus if the falling time does not exceed 16 seconds, the probable error introduced by the stop-watch will be less than $\pm 5 \times 10^{-5}$. The corresponding probable error curve for a watch split in fifths of seconds is obtained by doubling the height of each ordinate.

That drop size can be controlled so that the determinations will fall within this stop-watch error, is shown by the fact that numerous series give probable errors (circles) which coincide with this curve. A quantitative estimate of the significance of the drop size error has been gained by varying the drop volume $\pm 5$ per cent. This is at least five times what the average accidental variation should be.

These drop size tests were made at three different falling times, 21, 37, and 70 seconds respectively. At 21 seconds the variation from the standard size drop computed for a $\pm 1$ per cent change in size, was $\pm 3 \times 10^{-5}$. The corresponding variation for 37 seconds was $\pm 1.6 \times 10^{-5}$ and for 70 seconds was $\pm 0.7 \times 10^{-5}$. The results are indicated in Fig. 3 by crosses, and the broken line which connects them appears to intersect the curve of stop-watch error at about 18 seconds. Drops falling at this rate or faster can easily be held within the stop-watch error. Each circle represents the probable error of a single drop, whereas the location of each cross was calculated from the mean of a long and constant series, and hence is without significant error.

The probable error of a single drop at falling times greater than 18 seconds would always lie near the broken line except for one other recognizable source of error,—variation of temperature in the course of any series. We find some correlation between low error and stability of temperature, and since each change of $\pm 0.01^\circ$C. alters the density of the XBB nearly $\pm 1 \times 10^{-5}$, temperature variations afford ample explanation of why many series of drops requiring several minutes to test, give larger variations than those due to drop size only. To render the temperature error negligible in practice, it is necessary to drop the standard and unknown drops in close succession.
Error of the Method.—We have tried to make our procedure accurate to one point in the fourth decimal place. The probable error of the method must conventionally be from one-half to one-third of the desired sensitivity. That is, it must, in the present case, lie below the long dash line in Fig. 3. To give the probable error of the method corresponding to any determined probable error, whether of a single drop or of the mean of several drops, the latter must be multiplied by 1.4 ($\sqrt{2}$). This is because we have to do with an accumulation of two probable errors of equal size (of blood drop and standard drop).
Applying these principles of calculation most of the determinations in Fig. 3 are seen to attain the desired accuracy. Since the single drop errors were calculated from series of 4 to 9 drops, the probable error of the mean of each series is one-half to one-third as great. The sensitivity of the method is thereby doubled or trebled.

It is clear that with ordinary care and fairly slow drops density differences amounting to $1 \times 10^{-4}$ can be easily distinguished.

**Broader Application of the Method.**

From its sensitivity as shown above it can be seen that our procedure affords a very delicate method for determining the concentration of various solutions. These need not be watery solutions such as blood and serum but may be oily substances dissolved in bromobenzene or some other fat solvent. Such solutions must of course be dropped through a watery solution of appropriate density. The procedure outlined below will enable one to determine with very little effort the curve of drops falling through any one of a variety of substances. For we have found that a similar logarithmic relationship holds whether the contents of the tube be XBB pure, or made very viscid by the addition of petroleum oil, or (on the aqueous side) saturated zinc chloride or saturated potassium carbonate solution.

The accuracy can apparently be increased as far as desired by controlling the temperature rigidly and using very slow drops. Whenever the probable error in density is reduced to $\pm 1 \times 10^{-5}$, (see Fig. 3) this, computed from a drop measuring exactly 10 cmm., means that 2 drops can be distinguished whose difference in weight is less than $3 \times 10^{-4}$ mg. It would take $4 \times 10^{-4}$ mg. of sodium chloride to produce such a density change. But since we have further reduced the probable error in a carefully controlled series of slow (4 minute) drops to less than $\pm 5 \times 10^{-7}$, it follows that the method can be developed into a gravimetric procedure for distinguishing between quantities of known soluble substance at least as small as one or two hundred thousandths of a mg.

**Calculation of Results under Unusual Conditions.**—It remains to be explained how the results can be calculated when an unusual fluid is employed as dropping medium, when extremely slow drops
are to be studied for the sake of increased sensitivity, or when rigid compliance with all the specifications is not feasible.

From measurements of the falling times of a set of standard solutions of known density, a straight line can always be obtained on a logarithmic background as follows: The mean falling time of the slowest series which can be read is plotted against the corresponding apparent density difference as given on the standard alignment chart. The falling times of the other standards are then plotted against the density differences obtained by correcting the first ordinate plotted by the known differences between standard densities. This procedure should yield a straight line if the pipette, tube, and XBB mixture conform to the specifications we have outlined above. If, however, the curve obtained is not linear, it may be straightened out by raising or lowering all the points by a common density difference easily found by inspection. It is necessary to straighten the curve so that exact interpolations may be made, as well as to afford a basis for an alignment chart. It is to be clearly understood that this arbitrary correction is automatically cancelled in practice each time the apparent density difference of the standard is subtracted from that of the unknown. When a straight line has been obtained whether empirically or by correction, it must be compared at two or more points with the alignment chart. If it does not agree, it may be substituted for the latter as long as the same conditions, including room temperature, are continued. If work must be done at other temperatures, a new alignment chart similar to Fig. 1 can be constructed. If extreme accuracy is essential it is always safer to calculate from the curves drawn directly on a logarithmic background. These suffer no displacement with expansion or contraction of the paper due to changes in atmospheric humidity,—a real drawback to the accurate use of alignment charts.

**SUMMARY.**

A 10 cmm. drop of fluid is timed as it falls over a distance of 30 cm. through a mixture of xylene and bromobenzene in a tube of exactly 7.50 mm. bore. Its falling time is compared with that of a 10 cmm. drop of standard K₂SO₄ solution of known density. By the use of an alignment chart correcting for room temperature
it is possible to calculate the unknown density with an accuracy of $1 \times 10^{-4}$.

BIBLIOGRAPHY.

THE FALLING DROP METHOD FOR DETERMINING SPECIFIC GRAVITY
Henry G. Barbour and William F. Hamilton


Access the most updated version of this article at http://www.jbc.org/content/69/2/625.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/69/2/625.citation.full.html#ref-list-1