XXVI. METHODS OF MEASURING THE OPACITY OF LIQUIDS.

By J. HOLKER.

From the Department of Pathology, University, Manchester.

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PART I.

THE writer has long been of the opinion that, given a suitable and sufficiently accurate method of measuring the opacity of a liquid, it would be possible not only to study more closely than hitherto the mechanism of serum reactions, but also to evolve methods of importance in practical diagnostic and therapeutic pathology.

As none of the several available methods of measuring turbidity was suitable for the investigation proposed, it was necessary to devise one. In the first form of apparatus, which was worked out in 1918, it was decided to use the loop commonly employed in bacteriological routine for making sub-cultures. The loop itself was bent at the end of the wire at a right angle and the shaft was fixed to a sliding micrometer provided with a scale and vernier. In this way the depth of the loop below the surface of the emulsion at which it just disappeared from sight could readily be determined. This method was, however, discarded as not being sufficiently accurate. Recently Gates [1920] has published exactly the same principle for standardising vaccines. Priority for this particular method must, however, be given to Don and Chisholm [1911] who used it in connection with water analysis.

In the second form of apparatus which is entirely new in principle and which is the one described at length in this article, the wire was fixed in a constant position, whilst the depth of the liquid under examination was made to vary. This opacimeter (see Fig. 1) consisted of a tube of resistance glass rather more than 250 mm. long and 20 mm. wide, along the side of which was etched a millimetre scale. The bottom of the tube was blown flat; at the side, about 20 mm. from the bottom, was a narrow tube provided with a small tap, so that liquid could be run off to any depth. Running across the flat bottom of the tube on the outside from left to right was a piece of wire of known diameter, determined by means of a screw micrometer, and supported by two small blobs of plasticine about 1.5 mm. in diameter. The wire used was one of the fine copper shreds which can be obtained from the flexible material used in connecting up a wall plug with a portable electric lamp. It was blackened by carefully passing it through the Bunsen flame. The opacimeter was supported by a simple metal stand round the bottom of the tube, care being taken that, when the apparatus was standing upright, the wire and

the plasticine were clear of the bench. When a sufficiently turbid liquid was placed in the tube, the wire could not be seen. But on turning the tap the liquid could be run off until the wire just appeared. The reading was facilitated by the presence of the blob of plasticine at each end of the wire. These blobs being relatively much larger than the wire became visible first, and therefore acted as signals for a careful approach to the end-point. The end-point having been determined, the depth of the liquid was read off on the millimetre scale etched on the outside of the tube, the bottom of the rather flat meniscus being

Fig. 1.

taken as the correct level. The reciprocal of this reading was taken as a measure of the opacity of the liquid. As the reading of the scale of the opacimeter varied from 20 to 250, the turbidity varied from $1/20$ to $1/250$, i.e. from 500×10^{-4} to 40×10^{-4} . In writing up results the factor 10^{-4} was always understood.

To provide for a suitable source of light, a sheet iron box was made 5 inches high, 5 inches from left to right and 6 inches from front to back. In the lower part of the back of the box was a hole to support an electric bulb. At a distance of 3 inches from the bottom of the box and just above the upper level of the bulb were two shelves, one on each side, to support a sheet of opalescent glass

2 mm. thick, which served to diffuse the light from the incandescent filaments of the lamp. In this way a beautiful light was obtained of slightly yellowish hue. The roof of the box was formed by a loose plate containing a hole somewhat wider than the opacimeter tube and directly over the brightest part of the opalescent screen. Over the hole the opacimeter was made to stand, so that the wire and plasticine blobs appeared very clear and black against the contrasting background. The front of the box was provided with a sliding door. In order to cut off all light other than that coming from the opalescent screen, a sheet-iron tube 35 mm. wide and painted with lamp-black was dropped over the opacimeter during the time a reading was made. Between the lamp and the wall plug were placed an accurate ammeter and a resistance, so that the current could be readily regulated to within 0.02 amp.

The principle of the method depends primarily on the fact that, under the conditions of the apparatus, only particles above a certain size scatter the light. Ions or molecules in solution are so small compared to the average wave length of the light used that their scattering power may be regarded as nil. Particles of the size of amicrons probably scatter the light to a very slight extent, submicrons more markedly and microns powerfully. Large particles cast a shadow.

The light entering through the bottom of the tube casts a shadow above the wire, so that it is convenient to speak of a "shadow" space and a "bright" space. In the bright space the intensity of the light diminishes with increasing depth probably by the logarithmic law $y = ke^{-ax}$, where y is the intensity of the light at the depth x of the suspension, k is a constant which measures the intensity of the incident light, a is a constant which depends on the number, size and shape of the particles scattering light, and e is the base of the natural logarithms. On the other hand, owing to the scattering of light from the bright into the shadow space, the intensity of light in the latter increases probably logarithmically with the depth up to a certain level. But any light thus transferred to the shadow space is subject to the same law as that in the bright space, so that there must come a level higher up at which the intensity of light in the shadow space approaches zero again. In other words the intensity of light in the shadow space passes through a maximum, and beyond the maximum it approximates more and more to the intensity in the bright space. On this hypothesis the curves representing the intensity of light at different depths are depicted in Fig. 2.

Since the determination of the end-point or depth of supposed equality involves a discrimination between two intensities of light, Weber's law applies, and there will be a minimum proportional difference of intensity below which it is impossible for the eye to distinguish the difference in the two spaces. Therefore if, at the end-point, a is the intensity of light in the bright space, and b is that in the shadow space, then a/b may be equal to, but cannot be less than, the minimal proportional difference of intensity.

When the intensity of the source of light is increased, other conditions

remaining constant, the intensity in both the light and shadow space at the original depth of supposed equality, will be increased in the same proportion. For example, if the incident light is doubled the ratio will be $\frac{2a}{2b} = \frac{a}{b}$, so that the end-point remains the same for variations in the intensity of the incident light. That wide variations in the candle power of the electric lamp have no effect on the readings is shown by the following experiments. A suspension was taken of liquid paraffin in water. The strength of the suspension was

Fig. 2. Diagrammatic representation of the theory that, at the depth of supposed equality of light in the shadow and light spaces, there is a minimum proportional difference in the intensity of the light in the two spaces, that is, the ratio a/b is a minimum. When the incident light is increased, the light in both spaces is increased in the same proportion, so that the ratio remains the same.

adjusted so that the readings were of a convenient height. The first reading was made with a 16 c.p. lamp as the source of light, and the resistance was regulated so that the light was a bright one. Under these conditions the reading was 126.2. Keeping to the same conditions but now using a 32 c.p. lamp, the reading was 125.7. Again using a suspension of washed red corpuscles in physiologically normal saline with a 16 c.p. lamp and a bright light the reading was 187.7, with a 32 c.p. lamp and a bright light the reading was 187.8. The very slight differences observed between the readings were well within the experimental error. The detailed results of the experiment are collected together in Table I.

Table I. Showing that wide variations in the candle power of the electric lamp used as the source of light, produce only negligible changes in the readings of the opacimeter. The dull light was produced by currents of 0.26 and 0.42 amp. for the 16 and 32 c.p. lamps respectively. The bright light was produced by 0.30 and 0.59 amp. respectively.

Luminosity of lamp as regulated by resistance coil	Depth of suspension at end-point							
	Liquid paraffin suspension				Red corpuscle suspension			
	A		B		C		D	
	16 c.p. lamp	32 c.p. lamp	16 c.p. lamp	32 c.p. lamp	16 c.p. lamp	32 c.p. lamp	16 c.p. lamp	32 c.p. lamp
Dull ...	119.7	120.7	210.7	212.0	101.7	102.2	176.8	177.2
Bright ...	126.2	125.7	222.7	222.2	106.7	105.7	187.7	187.8

The figures in Table I show however that it is advisable to keep to one lamp and to regulate it by means of the resistance so that the amperage is constant, because the colour, and therefore the luminosity of the lamp, varies with the heat of the filaments. Thus with a 16 c.p. lamp, which with an amperage of 0.26 gave a dull slightly reddish light, the reading of a suspension of red corpuscles was 101.7; with an amperage of 0.28 giving a moderately bright yellowish light, the reading was 105.2, with an amperage of 0.30 giving a very bright slightly yellowish light, the reading was 106.7. Similar results were obtained with a suspension of liquid paraffin.

Occasionally it was necessary to use different opacimeters during one and the same experiment. A preliminary test was therefore made to determine how far the readings varied with different opacimeters, the same suspension being used in each case. With six opacimeters made according to the pattern described, the readings of a sample of milk diluted one thousand times with distilled water were as follows: 127.0, 126.3, 126.4, 127.0, 126.2, 126.6. The mean of these was 126.6. The respective differences from the mean were + 0.4, - 0.3, - 0.2, + 0.4, - 0.4, 0.0, and the mean difference from the mean was 0.3 or a percentage of 0.2. Hence it was permissible to use any opacimeter made according to standard.

Again, it was necessary from time to time for more than one observer to take readings during an experiment. Of the following observers *A* had good visual acuity, *B* moderately good, and *C* poor, though corrected to some extent by glasses. Using the same opacimeter the following results were obtained:

Suspension	Mean of all observers' readings	Percentage difference from the mean of each observer's readings		
		% <i>A</i>	% <i>B</i>	% <i>C</i>
No. 1	52.7	+ 0.6	+ 0.4	- 0.8
2	109.9	+ 0.6	+ 0.1	- 0.6
3	185.8	+ 0.1	+ 0.6	- 0.7
Average percentage difference from the mean		+ 0.4	+ 0.4	- 0.7

It is to be pointed out that the observer *C* had not only weak eyesight but was unfamiliar with the apparatus at the time the above readings were made.

If two or more observers frequently work together a factor for correction of their readings is easily calculated. Suppose the average percentage differences from the mean are + x , - y , + z respectively

$$\frac{\text{Corrected reading}}{A's \text{ observed reading}} = \frac{100}{100 + x}$$

$$\frac{\text{Corrected reading}}{B's \text{ observed reading}} = \frac{100}{100 - y}$$

and so on. Therefore the respective factors for correction are

$$\frac{100}{100 + x}, \quad \frac{100}{100 - y}, \quad \frac{100}{100 + z}.$$

For example the correction factors for Preston (very good eyesight) and Ponting (good eyesight) who frequently worked together were 0.994 and 1.006 respectively.

That one observer could reproduce readings for the same suspensions with a high degree of accuracy was repeatedly observed. For example observer *A* obtained the following consecutive readings with a suspension of *B. coli* in normal saline: 105.0, 104.8, 105.0, 104.5, 104.5, 104.3, 104.2, 104.0, 104.0, 104.0. The mean of these readings was 104.4, and the highest, lowest and mean differences from the mean were 0.6, 0.1, 0.3 respectively. The measure of the accuracy was therefore 0.29 %.

For accurate work it is preferable to work with suspensions having a reading of not much less than 100, because at or above this level a difference of, say, one millimetre in consecutive readings produces only a slight degree of error. Usually the average of three readings was taken, but for very accurate work more readings were taken. The highest degree of accuracy is obtained by working in a dark room, but for practical work this was not found necessary. It has been found that beginners tend to run off too much and obtain too low a reading. To avoid this it is advisable to make a preliminary observation by running off quickly until the plasticine blobs appear, and then running off more slowly until the wire just appears. After refilling the apparatus, the height of the liquid should be adjusted at about 10 to 15 mm. above the preliminary reading, and the tap then very carefully turned on so that the delivery is in slow drops. One drop a second was satisfactory, and at this speed three drops corresponded closely to a fall of 1 mm. This method of making a determination of the end-point also avoided eye fatigue, as the average duration of a reading was only about 25 seconds. The end-point having been determined, the reciprocal was found either by the use of a slide rule or more conveniently from a table of reciprocals such as is found in Castle's tables [1917].

Occasionally the opacity of a liquid was so slight that the reading was greater than 250 mm. (the height of the tube). When this was the case the method described below was used. The stands of two opacimeters were cut so that the tubes could lie close together. The circular cover was replaced by a rectangular one passing over both tubes but having a partition down the middle to provide a separate compartment for each. The loose roof of the box with a circular hole was also replaced by one having a rectangular hole about the dimension of the bases of the two tubes. Into one tube was placed the liquid of unknown opacity, the tube being nearly full. Into the other tube was placed an emulsion of similar colouring to the unknown and having a reading somewhat less than 250 mm. As a general standard, a stable suspension was obtained by diluting a few drops of Angier's emulsion of paraffin with distilled water. Such a suspension with a reading of 200 had repeatedly been found unchanged at the end of the day. The two tubes having been filled with the respective liquids, the emulsion of known opacity was then run off until its opacity matched that of the unknown.

Then if x = required reading of unknown emulsion,

p = depth of unknown emulsion actually present in tube,

q = reading of known emulsion, the reciprocal of which is the measure of its opacity,

r = reading of known emulsion which matches the opacity of the unknown,

$$\frac{x}{p} = \frac{q}{r}, \therefore x = \frac{pq}{r}.$$

The reciprocal of x then gave the opacity of the unknown. This formula was tested by preparing known dilutions of emulsions whose readings came below 250 in the original. By this means a turbidity as low as 8.7 has been measured.

As in experimental work it was often a question of measuring the number of particles in a suspension, it became a matter of importance to test whether the ratio between the opacity and the concentration was constant, that is if $T/C = k$. As the scattering power of the suspension depends on the number, size and shape of the particles, one would expect that, provided the size and shape remained constant, the turbidity would vary directly as the number of particles. This was found to be the case. For example, a suspension of *gonococci* in physiologically normal saline gave the following results, 100 being taken as the concentration of the strongest suspension.

Concentration C	Opacity T	T/C
100	102.0	1.020
66.6	67.6	1.015
50.0	51.0	1.020
33.3	33.8	1.015

Similarly pure cultures of *Staphylococcus aureus*, *B. coli* and suspensions of washed red corpuscles obeyed the same law, so that once the concentration and opacity had been correlated, it was sufficient afterwards to measure the opacity in order to determine the concentration.

PART II.

The apparatus described in Part I of this paper proved very useful in measuring the opacity of suspensions of which the amount was not very limited. In serum reactions however the amount of material is usually strictly limited and as the author was anxious to study these reactions he was obliged to modify the apparatus.

In the new form the lighting arrangement is similar to that used in the first method, but the liquid whose opacity is to be determined is placed in a narrow tube 50 mm. long, and holding about $1\frac{1}{4}$ cc. As will be seen from Fig. 3 this micro-opacimeter is placed between the source of light and a macro-opacimeter containing a standard suspension. Before measuring the unknown suspension the reading of the standard is taken with the micro-tube containing distilled water only. Then a micro-opacimeter containing the

unknown suspension is substituted for the one containing the distilled water and the reading of the standard again taken. The second reading is lower than the first, and the lowering is in proportion to the turbidity of the unknown.

Let S = reading of standard when distilled water is interposed,

M = difference of readings of standard when distilled water and unknown respectively are interposed,

X = required reading of unknown.

Then $X/50 = S/M$,

$\therefore X = 50S/M$;

$\therefore \text{turbidity} = \frac{1}{X} = M/50S$.

In practice the standard was always adjusted at the beginning of the day to a reading of 200, so that the turbidity formula became $\frac{M}{50 \times 200} = M \times 10^{-4}$. As the factor 10^{-4} is always understood in turbidity determinations, the factor M , which represents the difference between 200 and the reading of the standard

with the unknown interposed, gives us at once without any calculation the opacity of the unknown.

The standard used was a stable one made by diluting a small drop of Angier's emulsion of liquid paraffin with distilled water. Such a suspension keeps constant throughout the day, and is found to have changed only slightly even on the following day. It was prepared fresh each morning.

The micro-opacimeter tube is made of Jena glass about 5.5 mm. wide. Pressed firmly against the ground edge of the bottom of the tube is a plate of optically clear glass. In this way any possible error from the use of a cement joint is avoided. The joint was found to be completely water-tight. Around the lower part of the tube is cemented a brass collar provided with a thread. Screwed firmly on to the collar is a brass cap which is pierced by a hole of definite diameter so as to allow a definite amount of light to pass up the tube. Inside the cap is a rubber ring between which and the tube lies the plate of glass mentioned. The upper edge of the tube is ground flat, and when the opacimeter has been slightly overfilled so as to produce a convex meniscus a loose optically clear glass disc is dropped on to the top of the tube. In this way the upper surface of the liquid in the tube is rendered quite flat. With the narrow tubes used it was found that the sharply curved meniscus, which is formed at the upper surface of the liquid, acted as a powerful lens, and considerably altered the apparent diameter of the shadow. Though the length of the micro-tubes was adjusted by grinding to as near 50 mm. as possible, it was finally determined with accuracy by means of a travelling microscope, and a factor for correction calculated for each tube.

The macro-opacimeter is also provided with a metal collar and cap holding a glass disc against the bottom of the tube. The macro-tube is rather longer than 250 mm. and 38 mm. wide. It was found advantageous to increase the width of the tube beyond that used in the first form of apparatus, as by so doing the meniscus becomes sufficiently flat to be negligible in its action as a lens in altering the diameter of the shadow. During a reading a blackened metal tube was dropped over the large tube in order to cut off side light. Similarly to cut off top light the metal tube was provided with a metal cap containing in the middle a short tube 45 mm. long, against the aperture of which the eye could be placed. During the reading the front of the lamp box was closed by means of a sliding door.

A further improvement on the method described in Part I was that instead of a separate wire being placed at the bottom of each tube, only one wire was used. This was placed between two optically clear discs of glass which were pressed together by two brass rims having apertures 10 mm. wide. In order that the wire might be steady and retain its position between the glass plates, two small blobs of plasticine were also pressed flat near the ends. These blobs of plasticine also acted as signals for the approach of the end-point. The metal cell containing the wire was supported in a shelf in a position just above the highest part of the opalescent screen and directly in line with the light passing

up the two tubes. A better method of producing the shadow would be to replace the wire by a "graticule" or black line of known diameter etched by a photographic process on to the glass disc.

PART III.

The apparatus described in Part II has proved exceedingly useful for studying the interaction of electrolytes, suspensoids and emulsoids, and especially for covering a wide ground with only a limited supply of material. It was felt however that a more perfect apparatus would be one in which the reading could easily be taken from either direction, that is, not only when the shadow just appeared as in methods I and II, but also when the shadow just disappears. Preliminary efforts were made with the apparatus described in Part II by connecting a gas analysis funnel by means of rubber tubing to the side tube of the macro-opacimeter. By raising or lowering the funnel the level of the standard could be moved in either direction at will. However, it was thought that a better method would be to use a plunger which could readily be moved up and down with a rack and pinion. The plunger consists of a hollow tube of brass on the bottom of which is pressed an optically clear plate of glass in the manner described in Part II. The height of the under surface of this disc from the bottom of the macro-opacimeter is registered in a millimetre scale on the side of the plunger. As a further refinement a fine adjustment has been provided so that the depth of the plunger can be determined with an accuracy of one tenth of a millimetre. The initial readings are first made by means of the coarse adjustment, so as to get a fairly accurate idea of the position of the end-point. The final readings can then be made with the fine adjustment. The fine adjustment is intended primarily for taking the readings of almost transparent liquids, and is especially useful therefore when the readings are from 0 to 20.

Still further refinements which the author hopes to try are the reduction of the width of the shadow and the use of a telemicroscope in the hollow plunger. The apparatus described in Part II proved exceedingly sensitive, but the refinements now suggested should render it still more so.

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