The Interference Microscope as a Refractometer for Liquids

By S. IVERSEN AND F. H. SMITH

(From the Department of Anatomy, Royal College of Surgeons of England, London, W.C. 2, and Research Laboratories of Messrs. Charles Baker, Croydon)

With one plate (fig. 2)

SUMMARY

Determination of the refractive index of liquids by the interference microscope was made possible by the use of a specially constructed holder for the liquid. This ensures a permanent lateral separation between the reference and the object areas and, as the depth of the object area is continuously increasing, a system of fringes is observed. The spacing of the fringes is a function of the refractive index of the liquid. By determining this spacing, a measurement of the refractive index is obtained. An accuracy of at least 0.001 in refractive index is obtainable by a single measurement.

The interference microscope makes it possible to measure the optical path difference, and thereby the refractive index, of two laterally separated features. In this paper its application as a refractometer for liquids is described.

In order to obtain a permanent lateral separation between the liquid and the reference area, a special liquid holder was designed. This consists of two semicircular glass plates whose diametrical surfaces are optically worked and maintained in close mutual contact. A quadrant of one of the plates is optically worked to form a wedge having an angle of approximately 10 degrees (fig. 1). The semicircular elements are cemented with Araldite to a glass base plate having a hole of about 5 mm radius concentric with the compound disk formed by the diametrically-contacted elements. This design makes it possible to avoid spoiling the diametrical interface with surplus cement, because the cement is confined to the perimeter of the compound disk. A wedge of liquid is obtained by placing approximately 0.1 ml of the liquid under a cover-glass on the disk.

The one flat half of the glass disk will serve as the reference area and the cut edge as a sharp boundary. As the depth of the liquid is continuously increasing along the wedge, a system of evenly spaced fringes will be observed when the glass disk is viewed through the microscope in monochromatic light (fig. 2, A). The thickness of the glass disk necessitates an objective of sufficiently long working distance; the 10X objective is therefore used. The length of the fringes will be 330 μ, which is the lateral dimension of the shearing area of this objective.

A fringe will appear whenever the optical path difference between the reference area and the liquid increases by one wavelength. If the layer thick-
ness of the liquid at the place of the first fringe is $t$, the next fringe will appear at the place where the layer thickness is $2t$, the next at $3t$, &c.: so the difference in layer thickness between two consecutive fringes is $t$. The numerical value of $t$ depends upon the difference between the refractive index of the glass $n_g$, and that of the liquid $n_m$. The greater this difference is, the smaller is the numerical value of $t$ and a greater number of fringes per unit length is observed. When $n_g - n_m$ decreases fewer fringes will appear and when $n_g = n_m$ no fringes will be seen. If we call the distance between two consecutive fringes $d$ and the number of fringes per unit wedge-length $NF$, we have

$$\frac{n_g - n_m}{t} \approx \frac{1}{d} \approx NF.$$  \hspace{1cm} (1)

Fig. 2, B depicts the appearance of the fringes for liquids of different refractive indices.

The measurement of the refractive index of a liquid is carried out either by measuring the distance between two consecutive fringes or, as we prefer, by counting the number of fringes per unit length, the fractional part of a fringe being measured by the use of the phase-measuring goniometer of the microscope. Fig. 3 shows the results of a series of measurements of liquids of different refractive indices. As seen from this figure—and from equation (1)—there is a linear relationship between the refractive index and the number of fringes per unit length.

As the refractive index of the glass will be known—1.5193 for this particular glass disk—and at this point the number of fringes is zero, the calibration of the refractometer is very simple. All that is needed is to determine the number of fringes per unit length for air ($n = 1.0000$) and then in a coordinate system, where the number of fringes per unit length is the ordinate and the

---

Fig. 1. Refractometer seen from above (A) and from the side (B).
FIG. 2
S.IVERSEN and F. H. SMITH
Fig. 3. Graph showing the relation between the number of fringes per unit distance and the refractive index of the medium.

refractive index the abscissa, to connect the two points by a straight line. Where greater accuracy is required, arithmetical computation is preferable. If \( A \) is the number of fringes in air per unit length, we have

\[
\frac{A}{n_g - 1.0000} = K, \tag{2}
\]

and if \( N \) is the number of fringes per unit length for a liquid of unknown refractive index \( (n_m) \), we have

\[
\frac{N}{K} = n_g - n_m, \tag{3}
\]

from which, as \( N, K, \) and \( n_g \) are known, \( n_m \) can be calculated.

As the true value is always unknown, the accuracy of a measurement is a matter of probability. As the degree of accuracy obtainable with this refractometer obviously depends upon the accuracy with which the fractional part of fringes can be determined, the standard deviation from a series of determinations of fractional fringes will be used as a measure for this probability.
Fig. 2, B shows that the width of the fringes increases as the difference between \( n_g \) and \( n_m \) decreases, and it might therefore be thought that the precision with which fractional fringes could be determined would depend upon the fringe width. That this is not the case is evident from results of measurements of fractional fringes in water and liquid paraffin. From 10 determinations in water the mean was found to be 0.4161 fringe with a standard deviation (s) of 0.01561 fringe and for liquid paraffin the mean was 0.1506 fringe with a standard deviation of 0.01214. As the variances of the two sets do not differ significantly \( F = 1.65; 0.7 < P < 0.9 \), it can be concluded that the fringe width has no effect upon the precision. Further, five sets of 10 determinations of fractional fringes for different liquids—at room temperatures ranging from 20°C to 25°C—showed, as judged by Bartlett's test, identical variances. These were therefore combined, yielding a standard deviation of 0.01428 fringe. As the mean value of a normal distribution is being considered as the best estimate of the true value, this numerical value of the standard deviation means that in 99 out of 100 cases a single measurement will deviate 0.001 or less in refractive index from the mean, or true value. This accuracy can be improved upon by repeating the measurement. If, for example, two determinations are carried out, either by repeating the measurement on the same unit length or by measuring on two unit lengths, the deviation from the mean would be 0.0007 or less in refractive index.

The accuracy obtainable with this instrument compares favourably with the accuracy obtained by other micro-methods (Bauer, 1949; Jelley, 1949), where the accuracy varies between 0.005 and 0.0005 in refractive index.

The work of one of the authors (S. I.) is supported by a grant from the British Empire Cancer Campaign.

REFERENCES
