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Standard Test Method for the Automated Determination of Refractive Index of Glass Samples Using the Oil Immersion Method and a Phase Contrast Microscope[1]

This standard is issued under the fixed designation E 1967; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method covers a procedure for measuring the refractive index (η_λ^t) of glass samples, irregularly shaped and as small as 300 µg, for the comparison of fragments of a known source to recovered fragments from a questioned source.

1.2 This test method does not include the measurement of optical dispersion or the measurement of refractive index (η_λ^t) at any other wavelength other than the Sodium D line (η_D^t). This method employs a narrow band pass filter at 589 nm, but other filters could be employed using the described method and allowing the η_λ^t to be determined at other wavelengths, therefore, also allowing for the dispersion value to be calculated.

1.3 Alternative methods for the determination of η_λ^t are listed in Refs (**1-5**).[2]

1.4 *This standard test method does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Summary of Test Method

2.1 A phase contrast microscope is employed with illumination at a fixed wavelength (nominally Sodium D) to magnify the image of glass particles while these are immersed in a silicone oil. The microscope is aligned to produce even illumination with maximum contrast and a video camera is attached to an eyepiece (the output of the image) to observe the immersed glass and measure the contrast of the image of the glass. The temperature of the oil is changed via a hot stage and an electronic temperature controller until the glass particles' image disappears. The temperature at which there is minimum contrast between the glass and the liquid then is recorded manually or electronically.

2.2 A microprocessor or other handling station, such as a personal computer, employs a video camera interfaced by appropriate software and hardware to view the glass fragments. These commercial electronics result in a digital count representing a preselected edge feature's contrast being determined. This edge or contrast measurement is updated with every frame of video as the temperature of the hot stage, oil, and sample are ramped up or down. The software automatically registers the match point by taking the average of the minimum contrast measurements for both the cooling and the heating cycles. This match temperature can be converted to η_D^t by reference to a calibration curve for the immersion oil previously created from the match temperatures obtained on reference glass standards. This calibration curve is obtained from reference glasses of known η_D^ts within the range of interest. This curve or its mathematical equivalent normally is stored within the microprocessor and is employed to determine the η_D^t of any glass of interest, whether it is a fragment of known origin or a recovered (questioned) fragment.

2.3 Precise control and measurement of the immersion liquid temperature is achieved by use of a microscope hot stage. A precision of 0.05°C for the hot stage is desirable, but a precision of 0.1°C is the requirement for interlaboratory comparisons.

3. Significance and Use

3.1 This technique modifies the sample, in that the glass fragment must be crushed, if it is too large, and immersed in oil for the analysis. Some sample handling, however, would enable the analyst to recover the sample in the crushed form, if necessary.

3.2 This test method is useful for accurate measurement of η_D^t from a wide variety of glass samples, where most glasses of interest have η_D^t in the range between 1.48 – 1.55 in η_D^t units.

3.3 The objective nature of the match point determination allows for a better standardization between laboratories, and therefore, allows for the interchange of databases between laboratories.

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[1] This practice is under the jurisdiction of ASTM Committee E30 on Forensic Sciences and is the direct responsibility of Subcommittee E30.01 on Criminalistics.
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[2] The boldface numbers in parentheses refer to the list of references at the end of this standard.

3.4 It should be recognized that surface fragments, especially from float glass samples, can result in η_D^t's measurably higher than fragments from the bulk of the same source (**5**).

3.5 The precision and bias of this test method should be established in each laboratory that employs it. Confidence intervals or a similar statistical quality statement should be quoted along with any reported η_D^t value. For instance, a laboratory may report that the error for the measurement, using a reference optical glass is 0.00003 units.

3.6 It should be recognized that this technique measures the refractive index of the glass at the match point temperature, which will be higher than ambient temperature, and thus, may give different η_D^t values from those obtained by other methods, which measure the refractive index at room temperature.

4. Apparatus

4.1 *Microscope*—A microscope outfitted for phase contrast and an appropriate objective (nominally 10× - 40×) with a long working distance condenser is employed.

4.2 *Temperature Control*—A hot stage connected to a control device with a working range of approximately 26°C to 118°C, having a minimum precision of 0.1°C is employed.[3]

4.3 *Imaging*—A video camera is required for the automated measurements and is mounted to an ocular or photography port of the microscope. The output from the camera is used for the image processing for automated match point determinations.

4.4 *Illumination*—A narrow band interference filter is employed as a monochromatic source. For Sodium D measurements 589 ± 5 nm with a band pass of 10 nm is appropriate. The intensity of the illumination is adjusted to give the brightest image possible, without overloading the video camera.

4.5 *Immersion Oils*—Silicone immersion oils having refractive indices within a specific range are required for the glasses under study and are calibrated with the necessary standard reference glasses of known η_D^t.

4.6 *Standard Reference Glasses*—A minimum of three reference η_D^t are used, when possible, for the calibration of each silicone oil to be used for the actual measurements.

5. Procedure

5.1 Prior to crushing the glass sample for the η_D^t measurement, one should be certain that the possibility of obtaining a physical match has been explored and other examinations requiring larger sample size, such as density have not been precluded.

5.2 Arrange the microscope for optimum illumination and phase contrast. To insure maximum contrast, make sure the annular illumination ring from the condenser is aligned properly with the phase contrast shift plate, which is located within the objective by viewing the superimposition at the back focal plane of the objective. This alignment can be accomplished a number of ways, the most convenient of which is the use of Bertrand™ lens or a phase centering telescope.

5.3 Calibrate the necessary η_D^t oil from a set of three oils represented by oils of approximately 1.50, 1.53, and 1.55 using reference glasses of known η_D^t to ± 0.00001. At least three glasses for each oil should be employed for the calibration. Once calibrated, the η_D^t of the oils can be plotted against the match temperatures to produce a calibration curve for each oil. The preprogrammed protocol within the automated system to perform this function can be used.

5.4 After using an appropriate cleaning technique, such as a deionized water and alcohol rinse followed by drying, crush a small fragment of the glass to be studied and deposit a small sample on a clean, flat microscope slide. Immerse this sample in the proper silicone oil and cover with a cover slip.

5.5 Place the covered slide onto the hot stage and focus the image. The phase ring alignment must be checked each time that a new preparation is made to ensure that the phase rings are in alignment.

5.6 Vary the temperature by ramping up, or down, past the match point and then cooling down, or heating up, past the match point. Record the match point temperature in both directions and calculate the average. With microprocessor controlled units, recording will be performed automatically. The match point is that point at which the contrast is at a minimum, which corresponds to the disappearance of the edge of interest.

5.7 Determine the η_D^t of the glass fragment measured by reading the η_D^t from the calibration curve (η_D^t versus match temperature) for the average match temperature. For the microprocessor-controlled units, this calculation is displayed and printed automatically. The η_D^t value will represent the η_D^t of the sample at the match point temperature. To obtain the η_D^t at ambient temperature the value must be corrected using the dn/dt for that glass. Note that this is not usually known for casework glass samples. The match point temperature must be noted in the final report.

5.8 Repeat the analysis to determine the precision of the measurement.

6. Standards

6.1 Check the system calibration periodically or prior to the performance of an analysis, as required.

6.1.1 A separate reference glass (control) of known refractive index, distinct from that used for the calibration, for example, NIST, Schott, Locke, should be used to verify the calibration curve.

6.2 Recalibrate the system any time that the control falls outside the acceptable parameters established by the laboratory or analyst for this procedure.

7. Precision and Bias

7.1 *Precision*—Using the microprocessor controlled determination of the match point temperature, a standard deviation of 0.00002 η_D^t may be expected on measurements of the separate reference glass over a 5 h period. Over a five day period, a standard deviation of 0.00003 η_D^t may be expected. The precision is independent of both the accuracy of the temperature measurement and the characteristics of the silicone oil.

7.2 *Bias*—Since the measurement of the sample η_D^t is a direct comparison to the standard reference glasses used, no

[3] Mettler Models FP502 and FP82 have been found satisfactory for this function.

bias exists. Bias may be introduced in interlaboratory comparisons due to the use of different standard reference glasses for calibration.

8. Keywords

8.1 glass comparisons; glass measurement; refractive index

References

(**1**) Miller, E.T., "Forensic Glass Comparisons," *Forensic Science Handbook*, Saferstein, ed., Prentice Hall, Englewood Cliffs, NJ, (1982) pp. 139–183.

(**2**) Association of Official Analytical Chemists Official Methods of Analysis (1990), "Characterization and Matching of Glass Fragments," (973.65), p.637–639.

(**3**) *Precision of GRIM Reference: Canadian Society of Forensic Science*, Vol 27, No. 3 (1994) pp. 203–208.

(**4**) Locke, J. "GRIM—A Semi Automatic Device for Measuring the Refractive Index of Glass Particles," *The Microscope*, Vol 35 (1987) pp. 151–158.

(**5**) Underhill, M., "Multiple Refractive Index in Float Glass," *JFSS*, 1980, Vol 20, pp. 169–176.