



NEWSLETTER

California Association of Criminalists

NEWSLETTER

Fall 1979

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EDITOR'S NOTE

Because little news has come across the desk since the last Newsletter (which was, admittedly, 2 months late) several of the usual sections do not appear in this Newsletter. We have however, made an effort to continue with the inclusion of informal communications, three of which are contained within. The next deadline is 1 December; keep the manuscripts coming.

FALL MEETING ANNOUNCEMENT

The Fall semi -annual seminar will be held 18-20 October at the Oakland Airport Hilton; by now everyone should have received at least one mailing regarding the seminar. Included on the schedule will be the final discussion of certification before the national ballot on the issue and the ethics proceeding which has been postponed at the last two meetings. A proxy form is enclosed in this mailing; if you cannot attend the seminar, be sure you give your proxy to an attending member.

IN MEMORIAM - RAY H. PINKER

The California Association of Criminalists, the field of forensic science and its many practitioners lost a special friend on June 11 of this year. Raymond H. Pinker was one of the earliest practitioners in the field of criminalistics in the United States. His first involvement with criminalistics was as chief chemist with Arthur A. Maas Laboratories in Los Angeles where he worked on investigations as a consultant to the Los Angeles Police Department. He was encouraged by document examiner Clark Sellers to take the position of chief forensic chemist in the L.A.P.D. laboratory. He took that position in 1929.

Ray brought to the fledgling field of forensic science professional and ethical standards which were not overly subscribed to at that time. He demanded a sound empirical approach and was not swayed by what the result of an analysis "should be". He did not tolerate the tendency to be so influenced in others.

In 1931 his experience in reviewing a case in which the "paraffin test" for gunshot residue was in error led him to examine this test critically during 1932. He found it to be unreliable. His first article, "The Preliminary Test for Blood" was published in 1934 in the Proceedings of the California Chapter of the International Association for Identification. He brought x-ray diffraction to the laboratory in 1937 and, with Spencer Moxely, developed a comparison microscope for bullet comparisons using a modified Osborne tintometer. He was interested in breath testing for alcohol and developed an instrument for this test in the late 1940s. With this history of involvement with the Los Angeles Police Department, it is not surprising that the refrain was "take it up to Ray in the Lab" when the objective was to "get the facts" in the "Dragnet" television series of the 1950s.

Ray's intense interest in the field involved him as a founding member of the California Association of Criminalists. Even after his retirement from the L.A.P.D. laboratory in 1965 he maintained an active interest in the organization. His frequent attendance at meetings was always apparent by the cluster of friends and associates who wished to share the warmth of his friendship and the stimulation of his experience and intellect. The fact that his sparkling and vivacious Ruby so exuberantly shared his time with us added another dimension to an already delightful experience.

Recognition of his many talents and contributions to the field came to him in the form of the Roger Greene Award from the California Association of Criminalists in 1969. This was the first award of recognition ever given by the Association.

After 36 years in the laboratory, Ray did not choose to sit back and simply enjoy his hobbies of photography, travel and electronics. He took on the task of continuing the development of the forensic science master's degree program at California State University, Los Angeles. He taught police photography, criminalistics for police officers and advanced criminalistics classes for laboratory personnel and students planning a career in forensic science.

With the breadth and depth of his contributions to the forensic sciences, to the Association and to his friends around the world it seems trite to say he will be missed, but he will be. I will miss him. I miss him already.

Charles V. Morton

CAC Newsletter
June 1979

THE CORRELATION OF DENSITY
AND REFRACTIVE INDEX IN GLASS - -
FORENSIC CONSIDERATIONS

David A. Stoney and John I. Thornton
Forensic Science Group
School of Public Health,,
University of California,
Berkeley, California, 94720

(Paper given at 53rd Semi-Annual Seminar, San Diego, May 1979)

Density and refractive index are well suited for forensic characterization of glasses: each property is extremely sensitive to changes in composition, measurement is non-destructive, and small fragments are sufficient sample sizes. When we measure both properties over a population of glasses, we observe a high correlation. This is illustrated in figure 1 as a plot of density versus refractive index for window glasses encountered in FBI casework (1). Electrostatic theory predicts a high correlation between these properties for any group of materials having similar composition (2) and as we can see, glass is no exception. Correlation causes a partial overlap of information when both properties are measured: after the first is measured we have a more restricted range for the other.

Earlier workers noticed this correlation, but agreed that there was sufficient deviation from direct correlation to make both properties useful for individualization. More recently the correlation has been described statistically. For window glass Dabbs & Pearson found a Correlation Coefficient of 0.93 (388 samples) (3). For the FBI data the Correlation Coefficient is also 0.93 (577 samples). The Correlation Coefficient squared gives the proportion of variation explained by a linear model; here 87% is explained. Citing this high correlation between density and refractive index, some investigators have concluded that the second property rarely provides additional discrimination (4), or that if both properties are measured only limited additional differentiation occurs (5). The correlation is high statistically, and in probability calculations it is crucial to recognize and deal with this correlation. But the high correlation does not in itself render the second property of limited use for individualization, or imply that it rarely provides additional discrimination.

It is helpful to make a distinction between Discriminating Power and the usefulness of properties for individualization. Discriminating Power, as I use it here, is the probability that a certain set of attributes will differentiate a pair of randomly selected samples. This probability describes a general discrimination over the population and was introduced to assist in a management decision of which tests to perform routinely (6). Small-down & Brown calculated the Discriminating Power of density and refractive index for a population of window glasses (300) (7). They found that when one property is measured accurately, the second will provide only a small increase in Discriminating Power. Since each measurement alone is very effective at distinguishing random sample pairs, the second property has, relatively, a diminished effect.

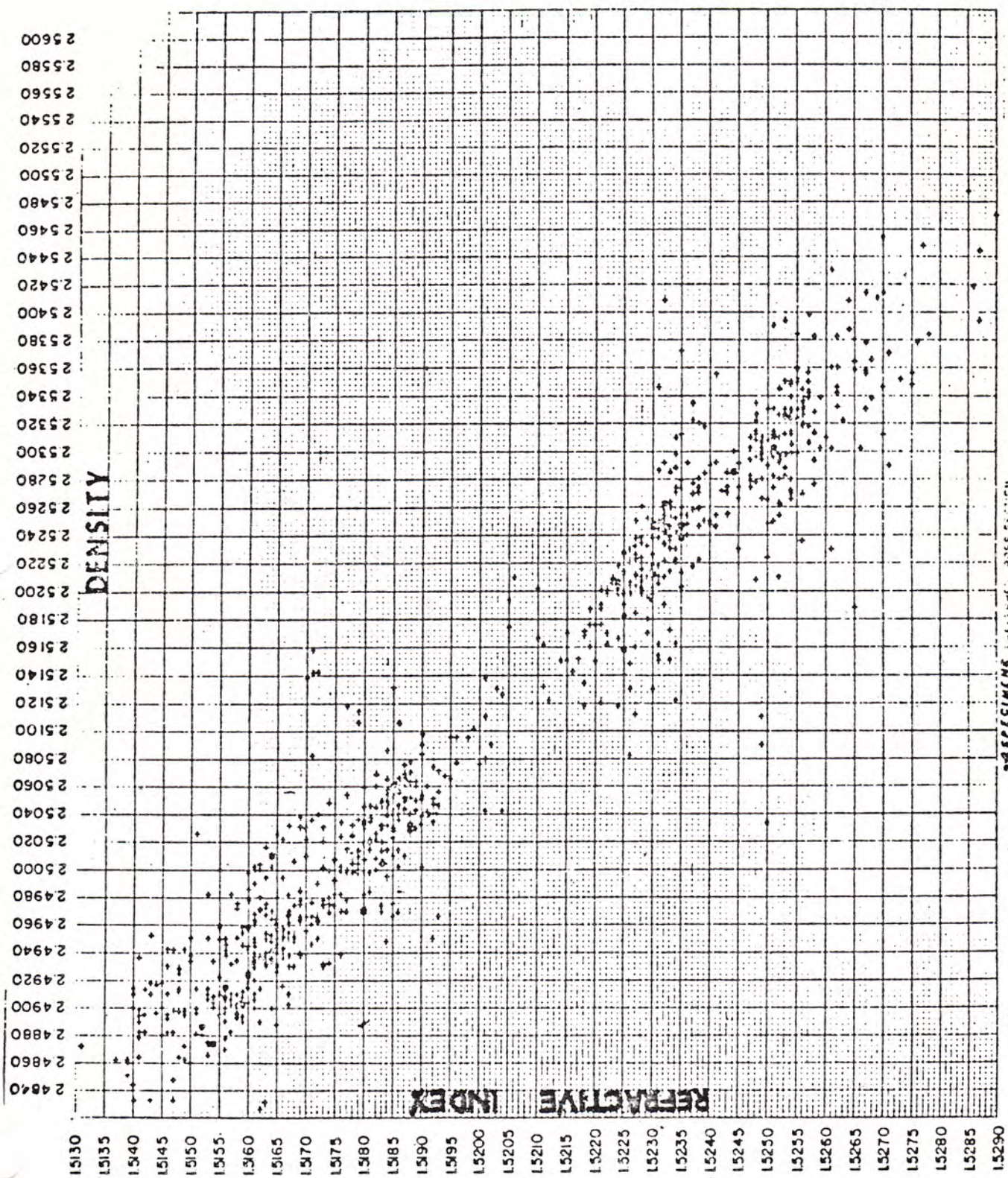


FIG. 1. No. 11. Density vs RH (stepwise)

In actual casework the situation is somewhat different; the measurements are made sequentially. If we differentiate our samples after one physical property is measured, we have little use for the second measurement. When differentiation is not achieved, we make further measurements. Beginning with the entire population as possible sources, a refractive index measurement eliminates the bulk of the possibilities. We are left with a restricted population, represented by a window on the plot of density versus refractive index (fig. 2). The width of the window will be determined by analytical precision. Instead of asking density to improve overall discrimination, we need to focus on the remaining possible sources; how well will density serve to reduce these? Although the correlation markedly reduces the range of most likely density values, many of the remaining possible sources may be eliminated by the second measurement, and the usefulness will vary according to the values of both properties and the precisions to which they are measured, but the width of this distribution insures that even for precise measurement of one property, the other is capable of providing significant additional discrimination.

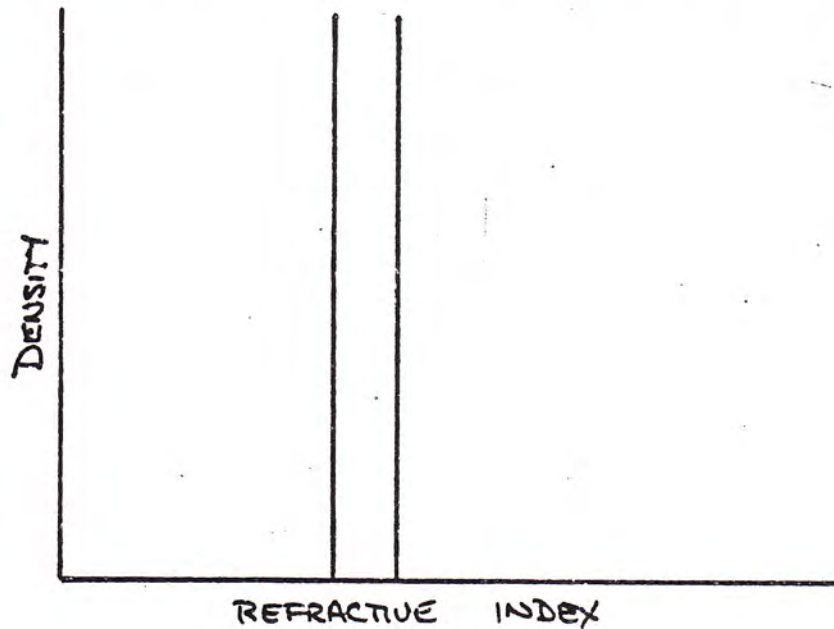


FIGURE 2

The distinction between this approach and Discriminating Power is a matter of perspective. Here we view the process sequentially and examine the additional discrimination independently of the first property. With Discriminating Power the two measurements are seen as a unit. The important point is that when a combination of properties are used for characterization we deal with an increasingly restricted population. The usefulness of additional properties needs to be evaluated with respect to this restricted population, rather than the total one. The total population is certainly not to be ignored; to evaluate the significance of a match in properties we must relate the final restricted population back to the total one.

Once both measurements are made, the possible sources appear as a rectangular region on a plot of density versus refractive index (fig. 3). Increasing analytical precision will reduce the size of this region and move us toward individualization. Eventually we will begin to measure within-item variation. To reduce the number of possible sources further we must examine the causes and character of this variation.

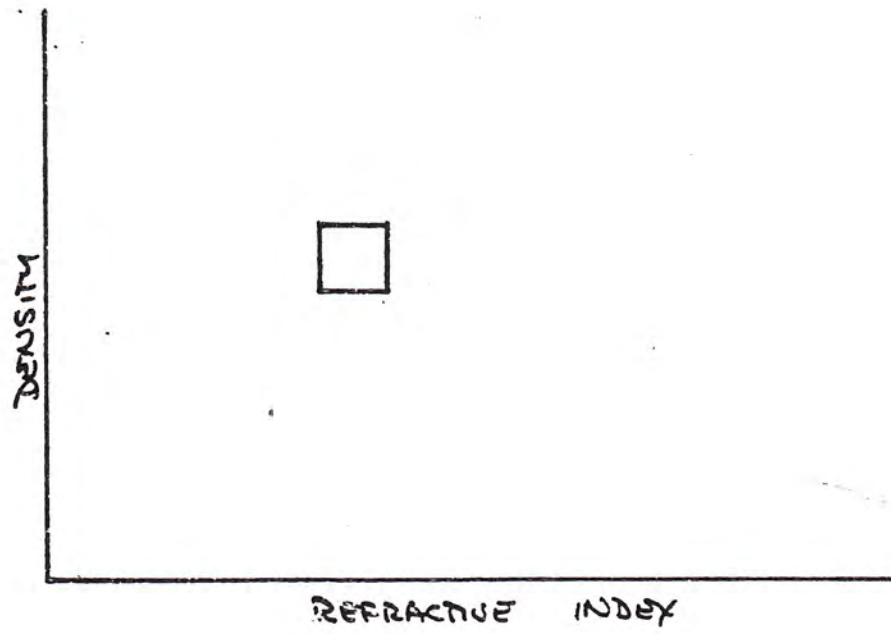


FIGURE 3

Density and refractive index in glass are primarily determined by chemical composition, but they are also affected by heat treatment.

The relationship between composition and physical properties has been the subject of extensive research. The simplest models assume the additivity of properties: each bulk property is described as a linear function of composition. The equation for additive properties is:

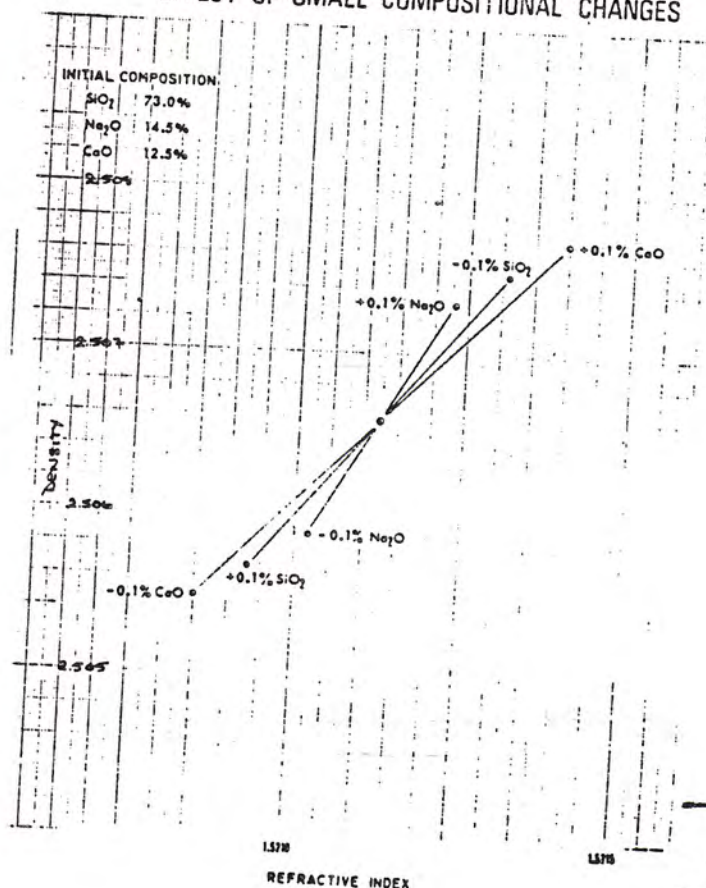
$$\text{Bulk Property} = aX_1 + bX_2 + cX_3 + \dots$$

where: a, b, c, \dots are constants, and
 X_1, X_2, X_3, \dots = mole percentages.

Both refractive index and specific volume are approximately additive with composition (8). The constants in these equations are derived empirically and have no rigorous physical or structural meaning (9). Two general types of constants are available: coarse estimates, applicable over wide ranges in composition, and more refined values for use in particular glass systems. Constants for commercial glasses are of this more exact type, and those derived by Huggins (10-12) are frequently used. More recently constants have been calculated from least squares analysis (13).

If the composition were accurately known, a number of physical properties could be estimated using the additivity relationships. For commercial glasses a more important use of these relationships is predicting the effects of small changes in composition. Figure 4 shows the effects on

FIG. 4
EFFECT OF SMALL COMPOSITIONAL CHANGES

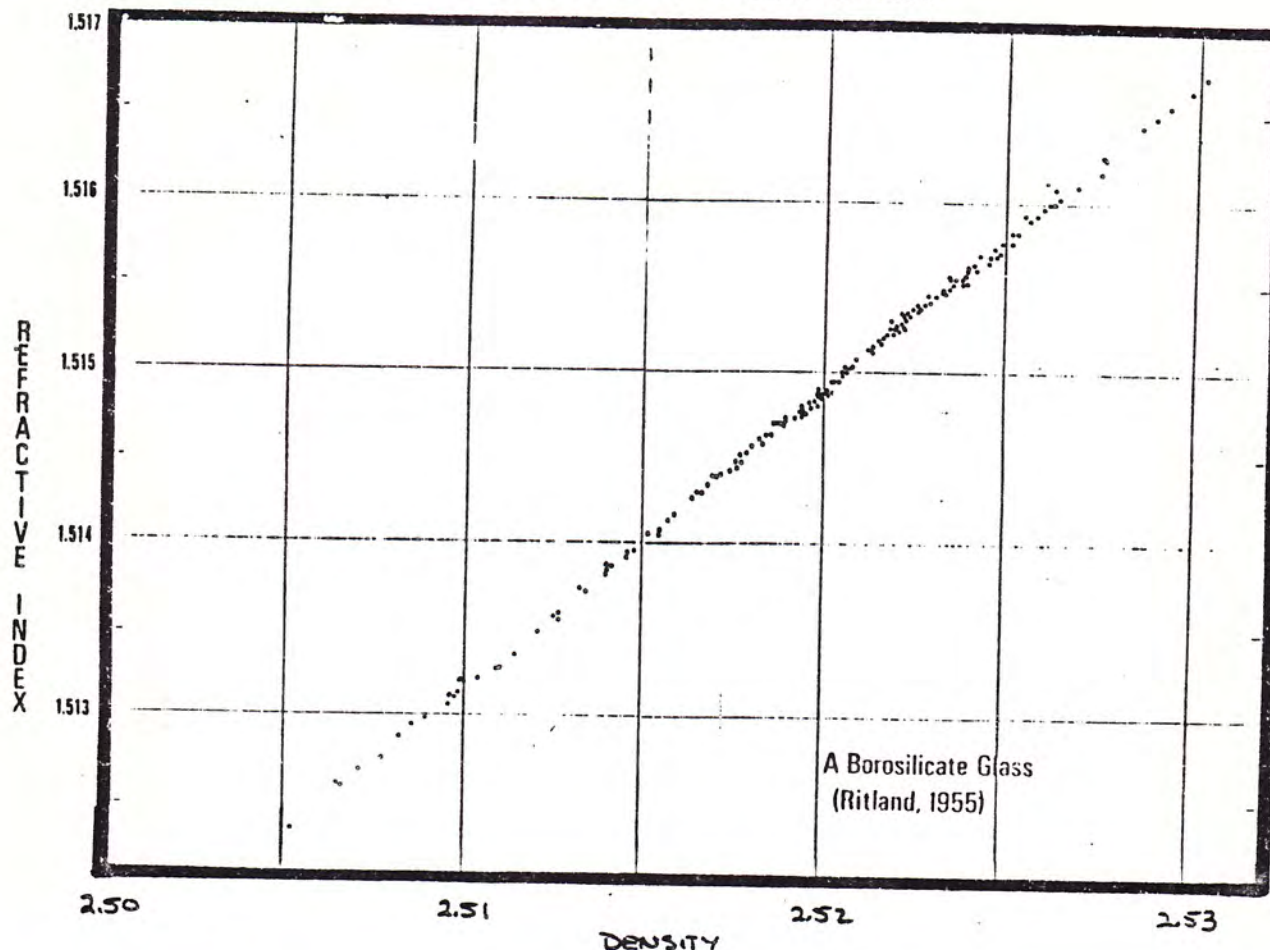


density and refractive index of a 0.1 weight percent deviation from an initial composition as shown. In the glass industry the weight percent of each component necessary to produce a unit change in density or refractive index is used for quality control purposes. The accuracy of these relationships is limited by the influence of heat treatment.

Glass of a particular composition will exist in different physical states depending on the heat treatment it has received (14). This variation is continuous and does not represent a phase change or discrete physical states. At different temperatures molten glass has different equilibrium states because the available energy determines what molecular processes occur. Lowering the temperature changes the equilibrium states and also increases the viscosity; molecular motion is inhibited and equilibrium requires a longer time to be attained. The rate of cooling determines how much molecular adjustment takes place before the arrangement is effectively "frozen in." Rapid cooling gives a looser arrangement with lower density and refractive index, whereas careful annealing produces the opposite effect: a denser glass of higher refractive index.

For a constant composition, changes in density and refractive index are very highly correlated over a wide range of heat treatment; Figure 5 shows data from a borosilicate glass. Over this range there is a constant of proportionality of 0.176. (15). The constant will vary with the particular glass system, but for the borosilicate and silicate glasses of commercial importance the value is approximately 0.17 (16).

FIG. 5
EFFECT OF HEAT TREATMENT



Within an item, then, variation may be caused by compositional heterogeneity or non-uniform annealing. The magnitude of these two effects will depend on commercial practice and so will vary with the manufacturer and type of product.

Intuitively we would expect heat-treatment differences in one item to be small. This is generally the case. Modern commercial annealing methods will usually result in differences between the outer and inner layers of glass on the order of 0.0001 in refractive index (17) (approximately 0.0005 g/cc in density). Tempering processes, on the other hand, produce severe temperature gradients in the glass and the refractive index spread increases to about 0.0004 (.002 to .003 g/cc in density) (18). Variation due to heating effects will also be higher when the glass is not annealed, as in the case of mineral wool (19).

A summary of work on within-item variation of physical properties of glass is provided in table 1. Because heating effects are limited for annealed products, much of this variation can be attributed to compositional heterogeneity. Refractive index ranges are close to 0.0002. The values for density spread are more erratic, but 0.0001 g/cc would be expected from the observed range in refractive index (20). Evett's data (21) deserves special mention (Figure 6). The standard deviation of refractive index for 230 windows was determined, giving a distribution of variation. The mode of 3×10^{-5} is useful for general considerations, but the distribution illustrates the need for a case by case evaluation of variability.

TABLE 1
INTRA-ITEM VARIATION FOR PHYSICAL PROPERTIES OF GLASS

Investigator (Date)	Glass Type	REFRACTIVE INDEX		Samples/ Item	Method	Variation X 10 ⁴
		Number of items				
Evetts (1978)	Window	230		10	immersion	Std. Dev., Mode .3
Andrasko & Maehly (1978)	Window	2		7	immersion	Ranges 2.3 and 2.1
Dabbs & Pearson (1970)	Window	2		25	immersion	Ranges 2.2 and 3.4
Ojena & DeForest (1972)	Headlamp	492		9	phase immersion	Mean Range 1.5 to 3.1
Malitson & Lechner (1975)	Headlamp	46	(ave.) 13		V-block	Mean Std. Dev. 0.5

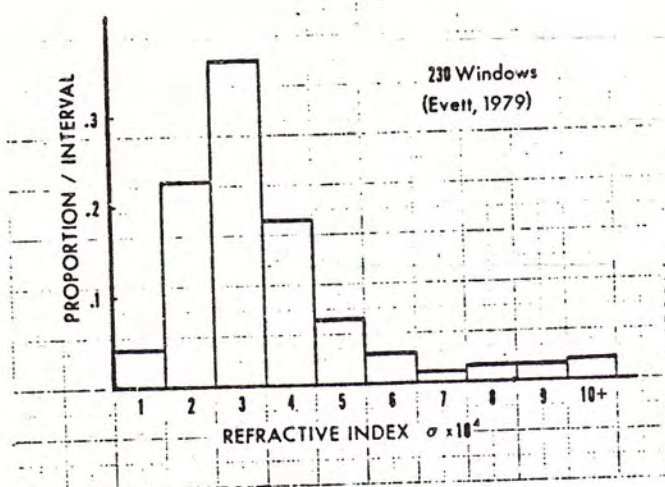
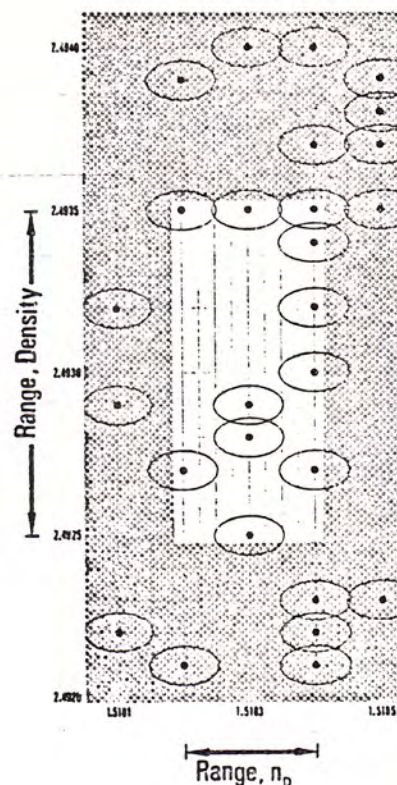
Investigator (Date)	Glass Type	DENSITY		Samples/ Item	Method	Variation X 10 ⁴ g/cc
		Number of Items				
Dabbs & Pearson (1970)	Window	2		25	sink-float	Range 3. to 6.
Crockett & Taylor (1969)	Safety	1		60	gradient	Std. Dev. 5.
Finch & Williams (1958)	Bottles	31		unk.	gradient	Range 3. to 23.
Ghering (1944)	Bottles	"extreme variation"			density centrifuge	Range 10.

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FIG. 6

WITHIN-ITEM VARIATION

FIG. 7
MINIMUM RECTANGULAR REGION

Let's return to the rectangular region containing glass sources yet to be distinguished from our evidence sample. Refined measuring methods diminish the area until we encounter variation within the sample. At this point we will observe a range in each property which describes a minimum rectangular area (Figure 7). We have potential to go further than this if we take into account the nature of within-item variation.

Glass samples from the same item will have nearly identical composition and heat treatment, so we can describe within-item variation using the relationships we have discussed. Figure 8 illustrates the variation we would expect. The small dark lines show the effect of small compositional differences and the red line, the effect of heating differences. Deviations from either cause occur with a general proportionality. Within the rectangular region defined by the ranges of density and refractive index, only values following this general proportionality are consistent with within-item variation. The rectangular region includes a substantially larger area, representing a greater number of indistinguishable samples.

To generate this more restricted region empirically, we need to measure both density and refractive index on individual fragments. If precision is coarse relative to the variation, there will be little advantage to making these pairs of measurements. Figure 9, a to d, shows the effect of decreasing analytical precision. In each illustration the lines show the expected variation in the glass itself, and the circles are the confidence intervals of measurement. Note that the ranges get larger with decreasing precision. In order for the ranges of intra-item variation to be accurately assessed the precision of measurement must be near the limit of available techniques and must be accurately known.

FIG. 8

Relationship of Ranges to
Intra-Item Variation

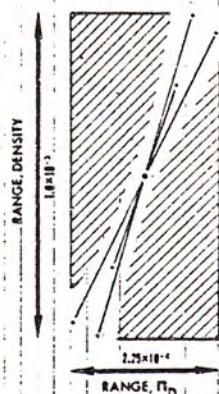


FIG. 9.

EFFECT OF ANALYTICAL PRECISION

DENSITY $\pm 1.5 \times 10^{-3}$
 $n_D \pm 2.5 \times 10^{-3}$



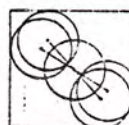
a.

DENSITY $\pm 1.5 \times 10^{-3}$
 $n_D \pm 5.0 \times 10^{-3}$



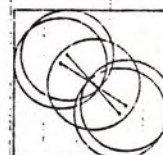
b.

DENSITY $\pm 2.0 \times 10^{-4}$
 $n_D \pm 1.0 \times 10^{-4}$



c.

DENSITY $\pm 2.0 \times 10^{-4}$
 $n_D \pm 1.5 \times 10^{-4}$



d.

Although there is a high correlation over the population, measuring one property does not determine the other. The range of likely values for the second property is restricted, but it is further divided by the second measurement.

Understanding the reasons for variation within glass should also allow for a more meaningful interpretation of small differences in physical properties. If differences are present between two fragments at the limit of detectability, do they occur with the proportionality expected for within-item variation? If so, further tests to confirm the differentiation might be indicated.

Measurement of both properties with precision on individual fragments has the potential to define within-item variation more accurately than ranges alone. In cases where intra-item variation is high this should have a practical application.

More generally, here is a case where within-item variation is not a barrier to further differentiation, which shows that there may be useful application for methods whose precision exceeds within-item variation.

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OBSERVATIONS
ON THE REFRACTIVE INDEX DIFFERENCE

AT THE SURFACE OF FLOAT GLASS

Stephen A. Shaffer
Fresno County Sheriff's Office

While doing a refractive index comparison on glass samples encountered in case work at the Oakland Police Department, I made an interesting observation. I was examining the refractive index of a small particle by the immersion method, using a liquid prepared by the mixture of M-bromo benzene and bromoform, monochromatic light supplied by a Beckman DK Spectrophotometer and a biological microscope at low magnification (4X obj., 10X ocular). Having adjusted the mixture of the liquid to approximate the refractive index of the glass, I was varying the wavelength of the light supplied by the monochromater while observing the immersed particle. I noted a disappearance of the Becke line for the one part of the particle at one wavelength, at which point the rest of the particle was distinctly visible. Readjusting the wavelength, I was able to match the previously unmatched areas of the glass with the liquid, at which point the previously matched areas were now distinctly visible. The edges in the particle which disappeared at one of the wavelengths were irregular and appeared to outline the bulk of the particle. However the edges matching at the second wavelength all appeared to be co-planar.

I had made some other observations on the known glass that was available in this case. I had noted distinct one-surface fluorescence in the glass when I illuminated it by ultraviolet light. This phenomenon is usually attributed to the float process by which much modern glass is manufactured. In this process molten glass is floated upon a bath of molten tin in one step of the manufacturing, apparently resulting in the diffusion of atomic tin into one surface of the glass. This surface will fluoresce brightly when illuminated by short wavelength ultraviolet light. It is known that the physical properties of glassy materials, including their refractive indices, vary continuously with chemical composition, so the dissolved tin in the surface of the float glass would be expected to have some effect on the refractive index at this surface. It is this effect that I believe I observed while viewing the immersed particle.

To check on my hypothesis I removed the particle I had been observing under the microscope, washed it, and examined it under the ultraviolet light. It showed, as I now expected, the fluorescence of float glass on one of its surfaces, a surface which appeared planar under the stereo. As a further check, I went back to the known glass, determined which surface showed the fluorescence, carefully removed small chips from this surface and from the opposite, non-fluorescent surface. These I checked with the ultraviolet light to verify that I had in fact obtained fluorescent chips from one side, and non-fluorescent chips from the other. I then examined these chips under immersion. My initial observation was confirmed in those particles from the fluorescent surface, while those from the non-fluorescent surface appeared uniform in refractive index.

CAP
Memorandum
June 1977

Subsequently I measured the refractive index of the known glass on an Abbe refractometer, measuring both surfaces of the known glass. I found a measureable difference in the values for the refractive indices on the opposite surfaces. (Since the Abbe refractometer measures the refractive index at the surface, I believe these measurements can be considered reliable and an indication of the chemical difference at the surface originally in contact with the tin.)

The question naturally arises as to what significance this adds or implication it has on the interpretation of glass comparisons in which the phenomenon is observed. It undoubtedly adds some significance to the refractive index data or information being gathered, but I am not prepared at this point to decide how much significance can properly be attached to this observation. Perhaps more on that another time.

A bit of reflection on this phenomenon gives rise to a few more thoughts.

Those among the readers who have pieced together broken windows (or other glass items) will probably recall that when a physical match is achieved between the broken edges of two pieces, the pieces may be slid back-and-forth past the matchpoint. Each time the match-point is passed, the pieces will "click" into place, and the fit is perfect, or nearly so, at that point. If the matching surfaces (Note that these are interior surfaces of the fractured glass) are examined, it will be found that the correspondence between the conchoidal and hackle fractures of the two pieces is nearly perfect. It is this correspondence that gives rise to the "click" and the positive feel of the match. It will usually also be noted that the match at the exterior surfaces of the glass is less perfect, these edges being more rough, sometimes having a feathered appearance. This is, I suspect, due to the nature of the crack propagation phenomena that go on in the body and at the surface of glassy materials. The relevant point here is that the vast majority of the microscopic particles which are sprayed out from a breaking glass object undoubtedly originate at or very near the surfaces of the glass. This would indicate that there is an appreciable probability that particles subsequently encountered on the clothing or tools of the one responsible for breaking the glassy object will include surfaces of the original article or window. When the glass involved in float glass, some of these particles should show the refractive index anomaly.

The float process of glass manufacture yields a product of flat glass suitable for use as manufactured as window glass. It may be manufactured in a wide range of thicknesses so may be encountered as single-strength, double-strength, or plate glass. But it can be further processed to yield automotive glass (either laminated for windshields or tempered for side or rear windows) which is no longer flat. Or, it can be tempered for a myriad of other uses, or mirrored.

Speaking of tempered glass, the tempering process involves the introduction of thermal stress in the surface of the glass which would be expected to introduce differences in the physical properties of the glass between the surfaces and the interior of the glass, much like the tin does in the float process. Briefly, the tempering process results in a sheet of glass in which the surfaces are under large and localized compressive forces, while the interior of the glass is under tensile forces. The compressive force in the surface of the tempered glass is responsible for the large increase in strength in the glass. I would expect this difference in relative stress from the surface to the interior of the glass to give rise to corresponding differences in the physical properties of the glass in these regions. However, it is possible that this

difference would be much less localized than the difference I observed in the float glass, being a continuous variation from one surface into the interior of the glass and back out to the opposite surface. I have not tested this hypothesis, so I don't know whether it will survive critical examination. In particular, I don't know the magnitude of the differences in stress resultant from the tempering, though presumably it is substantial, or the magnitude of the stress required to produce detectable difference in refractive index.

Anyone wishing to do further study on these phenomena should have no trouble obtaining suitable samples for study. Float glass is easy to recognize because of its marked one-surface fluorescence. For those individuals working in operational crime laboratories, one of the early Laboratory Proficiency Testing Program samples was float glass. Glass "B" of Proficiency Test Sample #4 was, I believe, that sample. Tempered glass is easy to recognize because of the way it fractures and ample supplies of this glass are usually as close as the pavement of the nearest busy intersection. If you have a laboratory collection of glass samples received in evidence, there is a very good possibility that among those samples you will find both float and tempered glass. If anyone has difficulty locating suitable samples, I would be happy to supply them. Drop a note to:

Stephen A. Shaffer, Criminalist
Fresno County Sheriff's Office
P. O. Box 1788
Fresno, Ca. 93717
(209) 488-3875

I would of course be most happy to see any further communications on this subject in future issues of The CAC Newsletter.

SYNTHESIS AND CLEAN-UP PROCEDURES
FOR 4-METHYLUMBELLIFERONE PHOSPHATE

Edward T. Blake¹
and
George F. Sensabaugh²

¹ Forensic Science Associates
1450 53rd St.
Emeryville

² School of Public Health
University of California
Berkeley

The most sensitive substrate for staining the products of the acid phosphatase 1 locus (ACP₁) is 4-methylumbelliferone phosphate. Methylumbelliferone phosphate¹ (MUP) is essentially non-fluorescent whereas its hydrolysis product, methylumbelliferone (MU) is intensely fluorescent. Hence the acid phosphatase isozymes are detected as fluorescent bands on a non-fluorescent background. It is apparent from these considerations that the sensitivity of the staining reaction will depend to some extent on the degree to which the substrate is contaminated with methylumbelliferone. Commercial preparations of methylumbelliferone phosphate vary a great deal in their purity and cost. Generally the more expensive preparations (\$100/gm) are less contaminated with methylumbelliferone than the less expensive products (\$11/gm). It is clear then that a simple clean-up procedure for the removal of methylumbelliferone from methylumbelliferone phosphate preparations would be valuable.

Methylumbelliferone phosphate is readily synthesized from methylumbelliferone and phosphorus oxychloride according to the procedure of Fernley and Walker (Biochem. J., 97, 1965, 95-103); several steps in their synthetic procedure provide for the removal of methylumbelliferone from methylumbelliferone phosphate using a solvent precipitation step. The synthesis and clean-up of methylumbelliferone phosphate is outlined below:

I. Synthesis of 4-Methylumbelliferone Phosphate

- A. To 8.5 g phosphorus oxychloride in 40 ml pyridine add 10 g 4-methylumbelliferone in 20 ml pyridine dropwise such that the temperature is maintained below 10°C. It is essential that the pyridine solvent be dry since the reaction between phosphorus oxychloride and water is violent. The reaction mixture should be stirred.
- B. After 30 min. add 100 ml ice cold distilled water to decompose the initial reaction product, methylumbelliferone phosphoryl chloride.

- C. Adjust reaction mixture to pH 7.5 with 10 N NaOH and remove solvents under vacuum with a rotary evaporator and heating mantle. The temperature of the solution should not exceed 65°C. The methylumbelliferone phosphate is in the form of the sodium salt at this stage.
- D. The resulting yellow powder is ground up in 30 ml acetone and extracted with 125 ml warm 80% methanol in water. The extracted material is yellow leaving a white precipitate which is discarded.
- E. Methylumbelliferone phosphate disodium salt is precipitated with 300 ml acetone. The precipitate should be extensively washed with acetone to remove methylumbelliferone. This is best accomplished by redissolving the precipitate in the methanol solution and reprecipitating with acetone. The precipitate at this step has a yellowish tinge and the consistency of freshly prepared butter. The powder can be dried by removing the acetone with diethyl ether.
- F. Methylumbelliferone phosphate disodium salt can be recrystallized from a minimum volume of hot 70% ethanol in water. The yield of the salt is approximately 6 g. and the crystals are slightly yellowish in color.
- G. To prepare methylumbelliferone phosphate as the free acid, dissolve the salt in water and submit to ion exchange chromatography using Amberlite IR-120 (H form) or some reasonable equivalent. Methylumbelliferone phosphate as the free acid elutes from the column in the void volume as a slightly yellowish liquid.
- H. Collect fractions containing MUP and remove water with rotary evaporator.
- I. The resulting white powder is dissolved in a minimum volume of hot ethanol and MUP is precipitated by dilution with diethyl ether. The product is collected as a white powder which is free of methylumbelliferone contamination. The yield is approximately 4 g of 4-methylumbelliferone phosphate.

II. Analysis by Thin-Layer Chromatography

- A. To check contamination by methylumbelliferone use Silica Gel G with acetone as solvent. MUP remains near origin and MU has Rf of about 0.75. Apply sufficient sample to detect small contamination.
- B. To check purity of MUP use cellulose plates with the organic phase of butanol (40), acetic acid (10), and water (50) as solvent. MU has Rf of 0.92 and MUP has Rf of 0.59. In addition there is usually a small phosphorylated contaminant at Rf 0.33.

To detect MUP spray dried plates with a solution of acid phosphatase in 0.1 M acetate buffer, pH 5.5. Two or three drops of seminal plasma in 10 ml buffer works quite well for this purpose.

III. Clean-Up Procedures for Commercial Preparations of MUP

- A. For MUP as the free acid, dissolve in hot ethanol and reprecipitate by dilution with diethyl ether until no additional precipitate forms.
- B. For MUP disodium salt, dissolve in hot 80% methanol and reprecipitate with acetone. Wash precipitate with diethyl ether and recrystallize dry powder from hot 70% ethanol.

In both cases the yield is about 95% and the resulting product is essentially free of methylumbelliferone contamination. Store samples frozen or refrigerated in a dessicator to prevent spontaneous hydrolysis of MUP.