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Dispersion Staining of Sugars

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KEYWORDS

Dispersion staining, mannitol, lactose, glucose (dextrose anhydrous), dextrose monohydrate, fructose, inositol, sucrose, maltose, high dispersion liquids, sugars, monosaccharides, disaccharides, carbohydrates, Christiansen effect, refractive index, Becke line, dispersion curve, annular stop, central stop

ABSTRACT

A variety of sugars is present in pharmaceutical products, illicit drugs, foods, explosives, and many other items commonly encountered by forensic laboratories. This paper begins with a few common spot tests for sugars, and a brief review of the dispersion staining technique and the theory behind it. Applications of dispersion staining are discussed, and the optical data for selected sugar compounds are presented in table form. Dispersion staining liquids suitable for sugar analysis are recommended, and a chart correlating dispersion staining colors to sugar identification is included.

INTRODUCTION

Determining the presence or absence of a sugar may be important, as in the case of a food sample that is supposed to be sugar-free or in identifying the components of an explosive mixture. It may be necessary to identify the particular sugar present, as in tracing a street drug to a specific source, or in differentiating one prescription tablet from another. Even when the sugar itself is not of primary interest, it may be useful to identify a sugar quickly in a mixture to assist with quantitative analysis or to screen a sample for contamination.

Sugars are carbohydrates and can exist as monosaccharides, disaccharides, or polysaccharides, some of which have very high molecular weights. Sugars can form chains that with increasing length form dextrin, starch, and cellulose, which together with lignin can ultimately form wood.

Classical chemical methods for detecting sugars (1) include the use of anthrone, which along with sulfuric acid and heat, turns blue in the presence of carbohydrates. The Molisch reagent is also used to detect carbohydrates. Barfoed reagent is used to detect monosaccharides; lead acetate is used for disaccharides. Fehling's reagent or Benedict's reagent is useful in testing for reducing sugars, which reduce cupric ions in the solution to a red cuprous oxide. Reducing sugars include all the monosaccharides as well as maltose. An aldose and a ketose can be differentiated using Seliwanoff reagent, and Bial reagent is useful for detecting a pentose. Iodine yields diagnostic color reactions with dextrin and starch, and starch grains can be speciated by examining the morphology of the particles in question.

Dispersion staining is an excellent technique for quickly detecting the presence or absence of a given substance or group of compounds, such as sugars. It is also a convenient method for detecting substances in a mixture, even when only a trace amount of the

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Table 1. Optical Properties for Eight Common Sugars

Sugar	α	β	γ	Birefringence	Optic Sign
Dextrose Monohydrate	1.515	1.528	1.557	Moderate	+
Mannitol	1.532	1.545	1.550	Moderate	–
Lactose	1.517	1.553	1.555	Moderate	–
Glucose (Dextrose Anhydrous)	1.528	1.556	1.565	Moderate	–
Fructose	1.558	1.558	1.561	Low	+
Inositol	1.553	1.562	1.566	Moderate	–
Sucrose	1.540	1.567	1.572	Moderate	–
Maltose	1.540 (ε)		1.550 (ω)	Low	–

substance is present. If an analyst is looking for a specific group of compounds, such as fibers, minerals, or sugars, and the dispersion staining properties are known, the sought-after information can be obtained rather easily.

MATERIALS AND METHODS

Dispersion staining is an optical staining technique; no chemical stains are employed. The sample is illuminated in a way that stains or colors the edges of a particle. The color observed when using axial illumination or an annular stop is the color of the wavelength of light at which the solid particle and the mounting medium have the same refractive index. For this phenomenon to occur, the solid and the liquid must have different dispersions of index, and they must have at least one index in common in the visible region of the spectrum. The occurrence of color along the edges of particles indicating that the solid and the liquid medium have the same refractive index somewhere in the visible spectrum is known as the Christiansen effect (2).

To achieve this effect, the substage aperture must be closed down so that the numerical aperture of the substage and the objective is not much greater than 0.1. The numerical aperture can be reduced significantly by removing the top lens of the substage condenser, the Abbe lens. Additionally, some objectives incorporate an iris in the lens itself that can be closed down as needed.

By using an opaque central stop, the axial light rays are blocked and only the annular rays passing through the specimen may be observed. These light rays bypass the objective lens except for those rays that are refracted by the specimen in a vertical direction. The wavelengths that are collected by the objective lens form the complementary color of the wavelength at which the specimen and the mounting medium have the same refractive index. In other words, the colors observed using a central stop are not the primary, but rather, the complementary colors of those observed using an annular stop.

To set up the polarized light microscope for dispersion staining, one should rotate the dispersion-staining objective (the objective lens with an annular stop, central stop, or iris) into place, remove the top lens of the substage condenser (Abbe lens), and the substage aperture should be closed until the dispersion colors are observed (annular stop) or until the field becomes dark (central stop). It is assumed that the polarizer is in place, but the analyzer should be out. A dark field is caused by the aperture being closed down around the central stop; not because the polarizers are crossed. The specimen is viewed in plane polarized light. Removing the polarizer may be helpful when analyzing mixtures, as will be discussed later in this article.

Dispersion staining can be used in a variety of ways to obtain much information about a sample. When placed in an appropriate medium, a single color around the edge of the particle in all orientations indicates a single refractive index and thus an isotropic particle, unless one is looking down an optic axis of an anisotropic particle. After obtaining a matching index (λ_0) in several refractive index liquids, a simple plot of the refractive index liquid indices versus the matching wavelengths will indicate the index at which the dispersion staining curve crosses the sodium D line (589 nm).

This should coincide with the literature index for the particle in question. Just as the Becke line is nor-

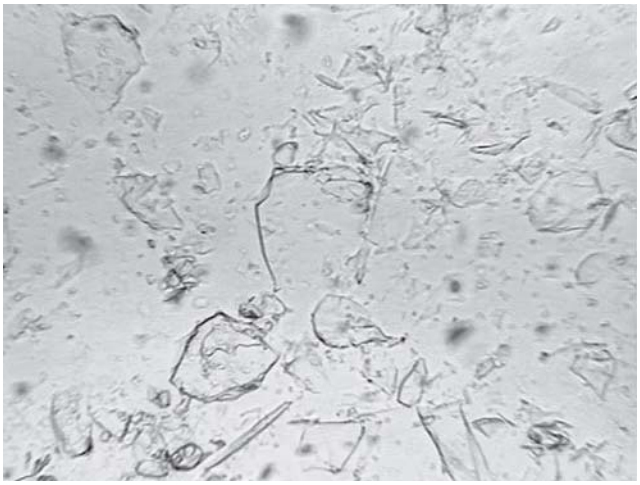

Figure 1. Fructose in plane polarized light.

Figure 2. Fructose in crossed polarized light.

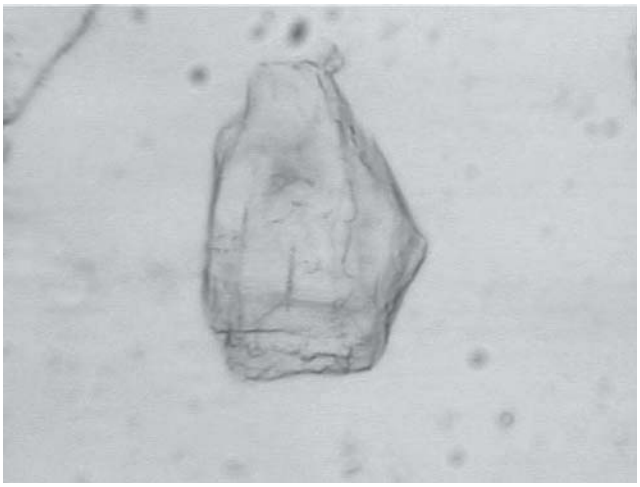

Figure 3. Equant glucose crystal.

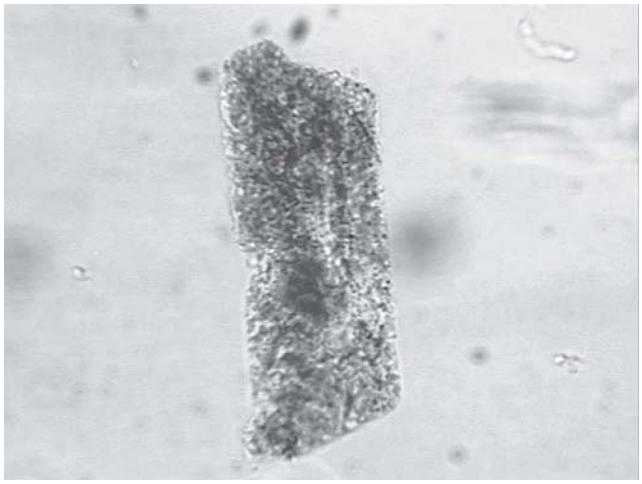

Figure 4. Elongated inositol crystal.

mally used to determine whether a particle has an index higher or lower than the immersion liquid, the dispersion staining color can be used for this purpose, and the specific color can be used to gauge the degree of contrast more precisely than the relief observed from a noncolored Becke line. If the Becke lines of a particle are white while using a central stop (or black while using an annular stop), this suggests that the index being observed is either higher or lower than the range of indices for which the liquid and specimen share a matching index.

When a sample exhibits at least two different colors in the same medium, this indicates that the sample is either uniaxial or biaxial and is therefore anisotropic. The dispersion staining color observed depends on the orientation of the crystal with respect to the vibration direction of the polarized light. For a uniaxial crystal, three orientations are possible. If one views the crystal down the optic axis, only the ω index will be observed. Otherwise, one extinction position will always exhibit the ω index, and the other extinction position will exhibit either ε or an intermediate index between ω and ε. Note that the ω index can be observed no matter what the orientation, so if a common color is observed for every particle upon rotation of the stage, the crystal is uniaxial. The highest and lowest matching wavelengths will correspond to ε and ω, with either being higher depending on whether the crystal is optically positive or optically negative.

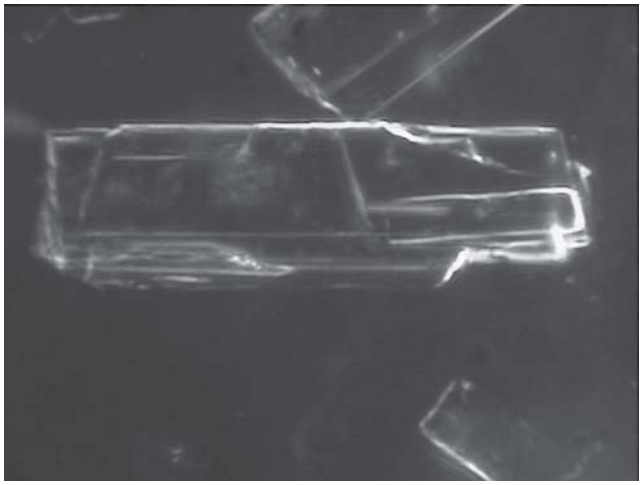

Figure 5. Elongated mannitol crystal in 1.520 refractive index liquid.

By plotting refractive indices versus matching wavelengths (λ_0), as with isotropic crystals, each index (ω and ε) can be determined based on where the dispersion staining curve crosses the sodium D line. If the ω index can be identified using the common color observed in all particles, then the ω index and the ε index can be specifically assigned to each dispersion staining curve. Once these assignments are made, the optic sign can be determined.

For biaxial crystals, randomly oriented crystals can yield many possible views. If one views a crystal down an optic axis, only the β index is observed. The crystal would behave as if it were isotropic. A second view would be any two of the three principal indices perpendicular to one another. One principal index and an intermediate index would constitute a third view, and finally, observing intermediate indices in all directions is possible. The highest and lowest matching wavelengths will correspond to (or at least approach) the α and γ indices. The higher matching wavelength corresponds to α; the lower matching wavelength to γ.

Because uniaxial and biaxial crystals exhibit multiple dispersion staining colors in plane polarized light, and because crystals may be oriented such that intermediate index views are seen, removing the polarizer may be helpful when examining a mixture. By removing the polarizer and viewing the dispersion staining colors in unpolarized light, each substance will behave as if isotropic, and only one color, the average dispersion staining color, will be observed for each component. The polarizer can always be reinserted to obtain additional information for a given compound.

Dispersion staining can be used to obtain optical data for a complete unknown, it can be used to detect submicrometer particles when the dark field (central stop) method is employed, or it can be used to analyze mixtures in a number of ways. The number of components in a mixture can be determined, one can determine the presence or absence of a given particle (even a single particle) in a complex matrix, and particle counting may be conducted for semiquantitative analysis.

If an analysis is limited to a fixed number of particles with known refractive index values, then dispersion staining provides a quick method for a given substance to be positively identified. This is the case with sugars. Some general optical properties for eight common sugars are provided in Table 1 (3,4).

Matching wavelengths for the selected sugars using both the annular stop and central stop methods are shown in Table 2, along with the corresponding colors. All matching wavelengths were obtained using Cargille high-dispersion refractive index liquids with the following n_D values: 1.530, 1.540, 1.550, 1.560, 1.570, 1.580 and 1.590. For the eight sugars selected in this study, the 1.560 refractive index liquid can be used to differentiate all but two, maltose and inositol; however, each of these can be confirmed using the 1.540 refractive index liquid.

In the 1.560 liquid, sucrose exhibits yellow and green colors with the annular stop. Maltose is the only one with a blue-green color in 1.540 with the annular stop. Glucose in 1.560 differs from mannitol using its annular colors and differs from inositol using its central stop colors. Lactose is the only sugar that exhibits blue and yellow colors for the annular stop in 1.560, while fructose is the only sugar that exhibits only yellow and orange with the annular stop in 1.560. Fructose and mannitol share similar dispersion colors with the exception of a red color for mannitol rather than orange. A second liquid is recommended for confirmation. Inositol in 1.560 is similar to glucose, but can be confirmed using 1.540.

Maltose is uniaxial, and fructose (Figures 1 and 2), which appears completely gray in crossed polarized light, is pseudo-uniaxial. All other sugars included in Tables 1 and 2 are biaxial. Glucose (Figure 3) and inositol (Figure 4) have similar optical properties and therefore exhibit almost identical dispersion staining colors; however, they are easily differentiated by shape alone. Inositol and mannitol (Figure 5) are elongated as opposed to the equant shape common to the other sugars.

CONCLUSIONS

In summary, sugars are present in foods, drugs, explosive mixtures, and many other samples submitted to the forensic laboratory. Dispersion staining provides a convenient and rapid method for identifying sugars without having to perform mechanical or chemical separations. By knowing the refractive index values of common sugars and the dispersion staining colors that each one exhibits in selected mounting media, rapid identification of each sugar can be achieved. For the eight sugars selected in the present study, dispersion staining colors can be used to identify each one using only two refractive index liquids, 1.540 and 1.560. High dispersion liquids are recommended. Additional liquids can be employed as necessary for confirmation.

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Table 2. Dispersion Charts for Eight Common Sugars

Dextrose Monohydrate

		1.510	1.520	1.530
Annular	n_1	Green	Yellow	Orange
	n_2	Violet	Blue-Green	Green-Yellow
Central	n_1	Red-Purple	Blue	Blue-Green
	n_2	Yellow	Orange	Purple
Wavelength (nm)		530	600	620
		420	480	560

Mannitol

		1.540	1.550	1.560	1.570
Annular	n = ↔	Green-Yellow	Yellow	Red-Brown	Dark Brown
	n = ↕	Blue	Green	Yellow	Red-Brown
Central	n = ↔	Purple	Blue	Light Blue-Green	Pale Blue-Green
	n = ↕	Golden Yellow	Red-Purple	Blue	Light Blue-Green
Wavelength (nm)		560	580	700	900
		460	530	580	660

Lactose

		1.550	1.560	1.570	1.580	1.590
Annular	n_1	Yellow	Yellow	Orange	Red-Brown	Red-Brown
	n_2	Blue-Green	Blue-Green	Blue-Green	Green	Orange
Central	n_1	Blue	Blue	Blue-Green	Light Blue-Green	Pale Blue-Green
	n_2	Orange	Orange	Orange-Red	Red-Purple	Blue-Green
Wavelength (nm)		580	590	600	660	700
		480	490	500	530	620

Glucose (Dextrose Anhydrous)

		1.540	1.550	1.560	1.570
Annular	n_1	Green-Yellow	Green-Yellow	Orange	Red-Brown
	n_2	Violet	Blue-Green	Green	Orange
Central	n_1	Purple	Purple	Blue-Green	Pale Blue-Green
	n_2	Yellow	Orange	Red-Purple	Blue-Green
Wavelength (nm)		570	570	620	700
		440	490	530	620

Fructose

		1.540	1.550	1.560	1.570
Annular	n_1	Blue	Green-Yellow	Orange	Red-Brown
	n_2	Blue	Green	Yellow	Orange
Central	n_1	Golden Yellow-Orange	Purple	Blue-Green	Pale Blue-Green
	n_2	Golden Yellow	Red-Purple	Blue	Light Blue-Green
Wavelength (nm)		460	560	630	700
		450	420	580	640

Inositol

		1.540	1.550	1.560	1.570
Annular	n_1	Yellow	Green-Yellow	Orange	Dark Brown
	n_2	Blue	Blue-Green	Green	Orange
Central	n_1	Blue	Purple	Light Blue-Green	Pale Blue-Green
	n_2	Yellow	Orange	Red-Purple	Blue-Green
Wavelength (nm)		610	570	640	900
		440	470	520	620

Sucrose

		1.540	1.550	1.560	1.570	1.580
Annular	n_1	Green-Yellow	Green-Yellow	Green-Yellow	Red-Brown	Red-Brown
	n_2	Blue	Blue	Green	Yellow	Orange
Central	n_1	Purple	Purple	Purple	Purple	Blue-Green
	n_2	Yellow	Pale-Yellow	Red-Purple	Blue	Pale Blue-Green
Wavelength (nm)		570	560	570	650	700
		430	400	520	580	610

Maltose

		1.530	1.540	1.550
Annular	n_1	Green-Yellow	Green-Yellow	Red-Brown
	n_2	Blue	Blue-Green	Yellow
Central	n_1	Purple	Purple	Pale Blue-Green
	n_2	Golden Yellow	Red	Blue-Green
Wavelength (nm)		560	570	700
		450	500	600