

SUGARS AND SUGAR PRODUCTS

Determination of Crystallinity of α -D-Lactose by Far Infrared Spectroscopy

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The far infrared absorption procedure for the evaluation of crystallinity of α -D-lactose is based on the oil-mull technique and the use of barium carbonate as an internal standard. Fixed slits, ordinate scale expansion, and very slow scanning rates are employed for analysis, and absorbance values are measured by the baseline technique. The method is applicable to samples such as whey solids and other dehydrated dairy products; however, the samples must be uniformly ground before analysis. For 10 test samples, the mean square error is 1.51% and the maximum error is about 2%. The time required for a single analysis is about 1 hr.

The degree of crystallinity of lactose in dehydrated dairy products is of manifest importance from the standpoint of processing as well as product quality (1-3). While various different crystalline forms of lactose can be obtained (3), the crystalline monohydrate of α -lactose is predominantly formed during processing and/or storage (1, 3). Because no satisfactory method appears to have been reported for the determination of crystallinity in the strict sense, the α -lactose content determined (after dissolution) by polarimetry is frequently taken as an indicator of crystallinity in the original solid sample (1, 2). A true evaluation of the degree of crystallinity in a solid product is, in principle, difficult because of 2 basic considerations: (1) Chemical methods are by their very nature not well suited to distinguish between different physical modifications of chemically identical specimens; and (2) physical methods for crystallinity determination depend on the state of dispersion of the specimen, i.e., particle size and preliminary grinding. Orientation of sample particles is also frequently of paramount importance. The latter statements hold true for both X-ray diffraction and optical methods. This

paper reports the results of an investigation to determine the feasibility of analyzing partially crystalline samples of lactose for crystalline α -lactose monohydrate by means of infrared absorption spectroscopy, using the Nujol-mull technique (4).

Spectroscopic Background

In the infrared region from 570 to 660 cm^{-1} (about 17.5 to 15 μm), crystalline α -lactose monohydrate exhibits strong absorption bands, while amorphous lactose, either in the α or the β form, results in an almost flat background. Because it is very difficult to evaluate exactly the amount of sample which is in the sample beam of a spectrometer in the form of a solid suspension, an internal standard is employed for quantitative measurements. Barium carbonate, which exhibits a sharp absorption band at 693 cm^{-1} , is suitable because of its inertness and favorable absorption characteristics. Figure 1 shows the absorption spectra of crystalline α -lactose monohydrate mixed with barium carbonate and of amorphous lactose in the 570 to 710 cm^{-1} region.

Quantitative infrared analysis is based on the Beer-Lambert law (4, 5), which is basically stated for liquid samples in the form (5):

$$-dI/dx = k c I \text{ or } A = abc,$$

where I = radiation intensity, x = pathlength, k = characteristic constant, c = concentration, A = absorbance, a = absorptivity, and b = sample thickness. If an internal standard is used and if the sample and standard do not absorb at the same frequencies, we can write:

$$\begin{aligned} A_1 &= a_1 b c_1 \\ A_2 &= a_2 b c_2 \\ A_1/A_2 &= a_1 c_1 / a_2 c_2 = k'(c_1/c_2) \end{aligned} \quad (1)$$

For powdered solids, the equation is not always linear because of optical inhomogeneity. The relationship can be expressed in a power series.

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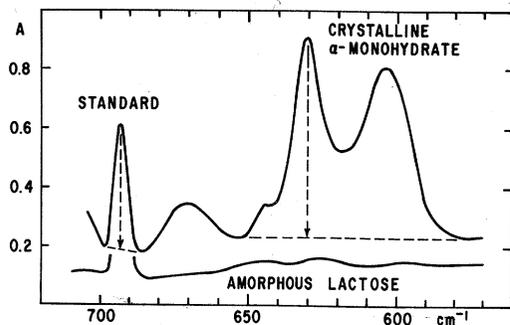


FIG. 1—Infrared spectra of amorphous lactose and crystalline α -lactose monohydrate with barium carbonate standard. Dotted lines illustrate the baseline method for absorbance determination. Absorbance is plotted vs. wave number.

Neglecting terms higher than quadratic, we obtain the following:

$$A_1/A_2 = k'(c_1/c_2) + k''(c_1/c_2)^2 \quad (2)$$

Subscripts 1 and 2 refer to sample and standard, respectively. For test samples the validity of the approximation can be tested by plotting

$$\left(\frac{A_1}{A_2} / \frac{c_1}{c_2}\right) \text{ vs. } \left(\frac{c_1}{c_2}\right);$$

k' is given by the intercept and k'' by the slope of the resulting straight line.

The nonlinearity has 2 principal causes: (1) If the particle size is very large, some radiation passes through the sample without encountering a representative amount of sample particles (6). The result is a "window" effect, illustrated in Fig. 2, and apparent negative deviations from the Beer-Lambert law, as illustrated in Fig. 3. (2) With small particle size, sample surface and reflection is increased (7), resulting in apparent positive deviations, unless the particle size approaches molecular dimensions. If the particle size is very small, both effects disappear, but so does the meaning of crystallinity. The constants k' and k'' thus depend on particle size and must be determined for a specific sample preparation; this sample preparation must permit consistent results and constant values of k' and k'' for a given type of analysis. For negative deviations (concave curves) k'' is positive; for positive deviations (convex curves) k'' is negative. In either case, k' designates the apparent absorptivity ratio (a_1/a_2) extrapolated to zero concentration of the sample ($c_1/c_2 = 0$).

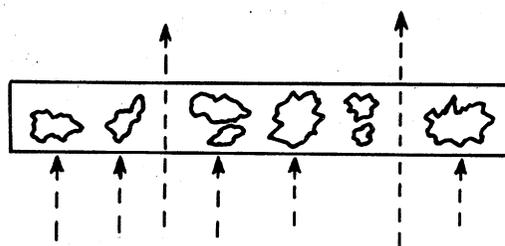


FIG. 2—Oversimplified illustration of the "window" effect resulting in too low absorbance values (high transmittance).

Experimental

Reagents and Apparatus

(a) *Crystalline α -D-lactose monohydrate*.—Obtained from Eastman Kodak Co. (white label).

(b) *Amorphous lactose*.—Prepared from the crystalline material by dissolving 2.5 g in 100 ml distilled water in the presence of a trace of ammonia to obtain an equilibrium α and β distribution. After ca 30 min, the solution was lyophilized and the amorphous nature of the residue was verified by infrared spectrometry (cf. Fig. 1). The material was preserved under dry nitrogen because traces of moisture frequently induce crystallization.

(c) *Spectrophotometer*.—Beckman Model IR with long wavelength interchange. The instrument was operated in the absorbance mode, with a mechanical slit width at 2.8 mm and a scanning rate of ca $5 \text{ cm}^{-1}/\text{min}$ (to avoid instrumental errors).

Determination

Test samples ranging from 10–100% crystalline material were prepared as follows: 100 mg total lactose, 50 mg BaCO_3 , and ca 0.3 ml mineral oil (Nujol) were ground 1 min in a specially designed grinding apparatus, with an electric stirrer motor rotating at ca 3000 rpm as measured by a stroboscope. The samples were spread between KBr plates separated by a 0.04 mm aluminum spacer.

Absorbance values for the standard and the crystalline material were evaluated by the baseline method (4); the baseline absorbance for the latter was corrected for amorphous material. (Because this correction is very small, the absorbance of the amorphous lactose was assumed to obey the Beer-Lambert law.) Linear ordinate expansion was employed to adjust the absorbance of the reference band to ca 0.4 absorbance units. Baseline points were chosen as indicated in Fig. 1. Figure 3 shows the absorbance ratio A_1/A_2 as a function of c_1/c_2 , expressed as per cent crystallinity. The constants k' and k'' were then determined by plotting $[(A_1c_2)/(A_2c_1)]$ vs. c_1/c_2 (cf. *Spectroscopic Background*). Values of $k' = 0.45$ and $k'' = 0.19$ were obtained. (Both constants are ratios

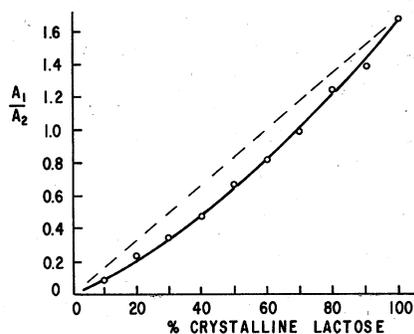


FIG. 3—Absorbance ratio of crystalline sample and carbonate standard plotted against per cent crystallinity.

and therefore dimensionless.) The continuous curve in Fig. 3 was calculated by equation (2), employing the obtained numerical values of k' and k'' . To obtain data reflecting the accuracy of the results, equation (2) was solved for c_1/c_2 , as follows:

$$c_1/c_2 = (-k' + [k'^2 + 4k''(A_1/A_2)]^{1/2})/2k'' \quad (3)$$

(The other solution of the quadratic equation leads to meaningless results.) Per cent crystallinity values were calculated by equation (3) for 10 test samples compared with the gravimetric data. Subsequently samples of dried whey solids were analyzed for crystalline α -lactose monohydrate and the results were compared with data obtained by the polarimetric method of Sharp and Boob (2). An allegedly sugar-free whey product was also examined for comparison.

Results and Discussion

A plot of spectroscopically evaluated crystallinity values, obtained by applying equation (3) to 10 test samples, vs. gravimetric input values was linear for samples having 10–100% crystallinity. The mean of the squares of the deviations of the spectroscopic measurements was 1.51% and the maximum error was 2.2%. The procedure thus appears reasonably accurate for the samples examined, considering that we are not evaluating the amount of a chemical entity but the degree of crystallinity. Although these results were obtained for samples containing only lactose, the method is applicable to products such as whey solids, because the other components, primarily proteins, do not show marked absorption in the 570–600 cm^{-1} range. Table 1 presents some results obtained for whey solids. It is interesting to note that the per cent crystallinity determined spec-

Table 1. Lactose crystallinity in dried whey solids

Property	Sample 1	Sample 2	Sample 3 ^a
Total lactose, %	67.3 ^b	69.2 ^b	~0
A_1/A_2	0.343	0.691	<0.01
Amt of crystalline monohydrate, %	30.2	52.9	<1
Crystallinity (spectroscopically), %	44.9	76.6	—
Excess α -lactose (by polarimetry), ^c %	49.5	81.4	—

^a Sugar-free commercial product, for comparison.

^b Colorimetric determination (8).

^c By the method of Sharp and Doob (2, 9).

troscopically is close to, but not identical with, the excess α -lactose determined by the method of Sharp and Doob (2, 9). It is frequently assumed that the α -lactose content of whey solids can serve as a measure of crystallinity (1–3). Our results suggest that this is a reasonable approximation, but an approximation nevertheless. The amount of excess α -lactose is a little higher than the amount of the crystalline form, as expected. Unfortunately, no method appears to have been previously reported which would determine crystallinity in the strict sense and could serve to verify the infrared procedure precisely. A sample of sugar-free whey concentrate was included to ascertain that other whey constituents (primarily proteins) do not interfere with the spectroscopic measurements.

The critical factor in the described method is sample grinding. Although consistent results were obtained, different grinding procedures could change the shape of the calibration curve and the values of k' and k'' . A considerable amount of empirical data is needed to establish the best procedure and eventually to establish a well defined method. In principle, the described approach can also be applied to crystalline β -lactose and crystalline anhydrous α -lactose. Both exhibit specific infrared absorption bands. Multi-component infrared analyses would, of course, be considerably more difficult (4, 5). The monohydrate of α -lactose is the most important form technologically. Once the procedure and the calibration curve have been set up, a determination can be accomplished in less than 1 hr.

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