Cold Stage for X-Ray Diffractometer Studies of Lower-Melting Polymorphs of Triglycerides

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ABSTRACT

Fabrication of an inexpensive cold stage attachment for an X-ray diffraction unit that gives satisfactory results to -21 C is described. X-ray diffraction data for several lower-melting polymorphs of cocoa butter are included.

INTRODUCTION

Polymorphism of triglycerides has interested investigators for over a century, and numerous experimental methods have been employed. X-ray diffraction powder studies demonstrated that the multiple melting point behavior was caused by the occurrence of different crystal forms of the triglyceride, distinguishable by characteristic X-ray diffraction spectra. Interpretation of the X-ray diffraction spectra, however, differed among investigators, and reviews by Lutton (1) in 1950 and Malkin (2) in 1964 illustrate the divergent viewpoints. More recently, X-ray single crystal studies, dielectric methods, infrared and nuclear magnetic resonance spectroscopy, and differential heating techniques have been applied to the problem of polymorphism. Chapman (3,4) has critically reviewed the various experimental methods and the state of knowledge of the polymorphism of glycerides.

Differential scanning calorimetry (DSC) (5) provides a technique whereby the triglycerides can be examined under dynamic thermal conditions. Thus, thermal events such as transitions from one polymorphic form to another, with or without partial melting, and the melting of particular polymorphs can be detected and quantitated as direct caloric measurements as these events take place. Identification of the polymorphs observed in triglycerides examined by DSC must be confirmed by X-ray diffraction spectra. Low-melting polymorphs are often unstable and, in some instances, occur below room temperature. To approximate conditions under which samples were examined in the DSC, a method of holding samples at a given temperature during X-ray scanning was needed. An inexpensive X-ray cold stage attachment was fabricated and provided a satisfactory substitute for the commercially available cryogenic diffractometer attachment.

The fabrication of the X-ray cold stage attachment and its application in obtaining X-ray diffraction spectra of selected polymorphs of cocoa butter at temperatures as low as -15 to -21 C are described.

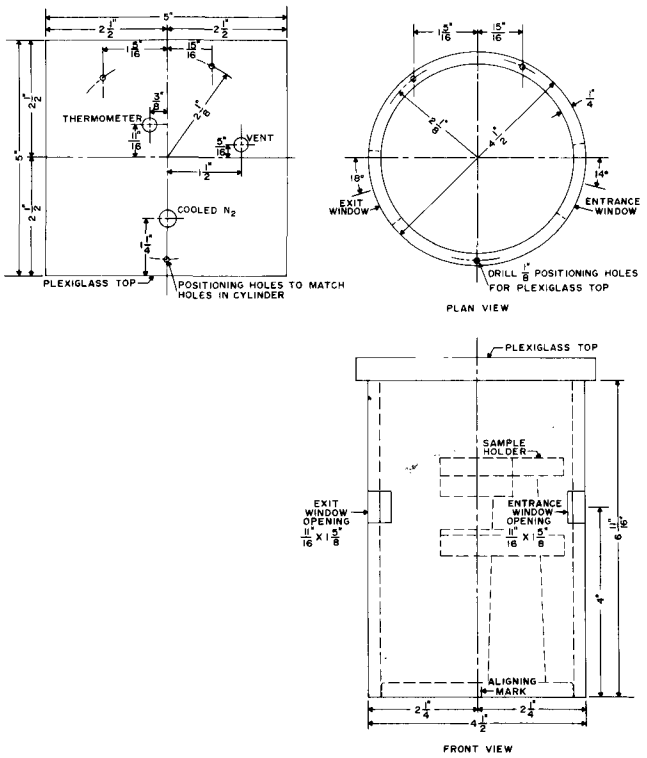

FIG. 1. Diagram of X-ray cold stage attachment.

MATERIALS

GE XRD-5 X-ray diffractometer unit with a CA-8S X-ray tube (Diano Corp., Industrial X-ray Div., Winchester, MA) was used for the diffraction studies. The instrument was set to scan the 2θ range from 2° to 40° at a rate of 2°/min.

The cold stage attachment shown in Figure 1, which fits over the base of the sample holder, was fabricated from a 6.75 in. length of type 40S heavy wall rigid polyvinyl chloride conduit, inside diameter ca. 4 in. with 0.25 in. walls (Carlon Prod. Corp., Div. Continental Oil Co., Cleveland, OH). The lower, inner edge of the conduit was machined to fit the 4 in. sample holder base. Two windows were cut in the wall of the conduit to accommodate the incoming and outgoing X-ray beam through the arc of the scan. The windows were covered with 0.00025 in. thick Spex X-ray film (Mylar) from Spex Industries, Inc. (Metuchen, NJ), taped in place to prevent moisture from entering the sample area and to contain the coolant. The Mylar film had little effect on the intensity of the X-ray beam. The long-term effect of X-rays on the film was not determined but is not important because the film is easily replaced. The cover for the chamber is a 5 in. square of 0.5 in. thick, clear plexiglass held in place by removable pegs (cut-off nails) that fit three asymmetrically placed holes in the cover and the chamber wall, which position the cover. Three holes were drilled in the center portion of the cover: one for the tube delivering the coolant to a position in front of the sample, a second for insertion of a thermometer behind the sample, and a third for a vent. Insulation of the chamber was provided by lining the inside with 0.5 in. thick urethane foam packing sheets, cut to fit. The base of the sample holder was insulated with several layers of corrugated cardboard. The coolant, dry nitrogen, was passed through 40 ft of 0.375 in. (outside diameter) copper tubing rolled into a 4 in. (inside diameter) coil, placed in a large Dewar flask packed with dry ice and acetone. A flow meter was placed in the line and the temperature inside the chamber was regulated by adjusting the flow rate. The rubber tubing (ca. 3 ft) delivering the cooled nitrogen from the copper coil to the chamber was insulated with standard refrigeration pipe insulation, taped in place. The Mylar windows fogged on the outside when the chamber temperature reached ca. 3 C; however, there was no increase in

fogging nor formation of ice crystals on X-ray scanning at -15 to -21 C.

If desired, the chamber could be modified for use at above room temperatures by substituting water for dry ice-acetone in the Dewar flask and heating with a probe-type heater suspended in the center space of the copper coil. The bath temperature could be regulated with a powerstat.

METHODS

At the start of a low-temperature scan, the cold stage attachment was put in place over the sample holder. The thermometer was inserted in the cover in the opening behind the sample holder and the coolant tube in the opening in front of the sample. The dry nitrogen flow was regulated with a valve on the nitrogen tank and monitored with a flow meter. Initially, a flow rate of 21 liters/min was used to cool the chamber. At this flow rate, temperatures decreased from 25 C (room temperature) to 10 C in 4 min. or from 17 C to -15 C in 19 min. The chamber was cooled to a temperature somewhat below that to be used for the diffraction scan and held a sufficient time to ensure that the sample holder had cooled to the desired temperature. On reaching the desired temperature, the flow rate to the chamber was regulated to maintain it. To maintain 19 C within the cold stage, the flow rate was 5 liters/min, increasing to 23 liters/min for -21 C. To insert the sample into the holder, the cover was removed, the sample was quickly inserted, and the cover was immediately replaced and the flow rate adjusted to maintain the proper temperature. After 2-3 min, the scan was started.

The cocoa butter to be scanned was prepared by several methods designed to obtain particular polymorphs. The untempered sample was heated above the melting point to ensure that the seed was destroyed, then poured into an X-ray sample holder held in the deep freeze (-32 C), solidified, and stored at -32 C until scanned. The partially tempered sample was heated until just melted, partially cooled, then a few shavings of solid tempered sample were added and stirred until a slight cloudiness occurred. The sample was then poured into an X-ray sample holder, held in the refrigerator (5 C) and stored at 5 C until solidified, after which the sample was tempered as desired.

Samples were transported to the X-ray area and stored until scanned in a well in the center of a container packed with crushed dry ice. This kept the sample at a sufficiently low temperature to avoid polymorphic transformations. Temperatures selected for the X-ray diffraction scans did not allow transformations during scanning.

DISCUSSION

Cocoa butter, one of the products being studied by DSC (6), was selected for X-ray diffraction studies using the above-described cold stage attachment. The interplanar spacings of four diffraction patterns for scans of cocoa butter with various degrees of tempering are given in Table I.

Wille and Lutton (7) identify six crystalline states for cocoa butter, I-VI, in order of increasing melting point, largely by X-ray diffraction. Their report also gives data on the stability of cocoa butter states at various temperatures. In discussing the polymorphism of cocoa butter, they prefer to use the term crystal states rather than polymorphs, reserving use of the latter term for pure triglycerides. Chapman (8) studied the polymorphism of cocoa butter using programmed temperature X-ray diffraction and DSC, utilizing the system of nomenclature of Wille and Lutton, which is used in this paper.

TABLE I

Interplanar Spacings of Cocoa Butter Diffraction Patterns[a]

A[b]	B	C	D
49.0 VS	49.0 VS	49.0 VS	33.3 VS
25.2 VW	32.7 VS	34.0 VS	22.1 VW
16.35 VS	16.35 M	16.35 M	16.35 W
9.82 VW	15.49 M	15.49 M	13.18 M
4.62 VW	12.99 W	13.18 W	10.77 W
4.25 VS	10.64 VW	5.34 VW	8.18 W
3.88 VW	9.82 VW	4.93 VW	7.19 W
	4.64 VW	4.62 VW	5.47 W
	4.35 S[c]	4.32 S[c]	5.21 W
	4.25 S[c]	4.21 S[c]	4.61 VS
	3.91 M	3.83 M	4.31 W
			4.01 M
			3.88 M
			3.77 M
			3.67 M
			3.39 VW
			2.59 VW
			2.40 VW

[a]Spacings in angstroms and relative intensity: VS = very strong, S = strong, M = moderate, W = weak, and VW = very weak.

[b]Tempering conditions and X-ray diffraction scanning temperature: A = untempered, scanned at 3.5 C; B = tempered 30 min at 3.5 C, 30 min at 10-11 C, scanned at 18 C; C = immediate repeat of scan B at -15 to -21 C; and D = seeded, tempered 1.5 hr at 23 C, scanned at 9.5 C.

[c]High points of a broad peak.

The untempered sample of cocoa butter (A), scanned at 3.5 C, is state II. State I rapidly transforms to state II below the scanning temperature. The partially tempered sample (B), scanned at 18 C, is state IV with some state III. When the sample was immediately rescanned at -15 to -21 C (C), complete transformation of state III to state IV had taken place. The seeded sample (D), tempered 1.5 hr at 23 C and scanned at 9.5 C, is state V. X-ray diffraction scans of tempered (unmelted) cocoa butter at 25 and 9 C (not given in Table I) were similar, but the scan at 9 C had somewhat higher and sharper peaks than that at 25 C, indicating a more crystalline state at the lower temperature.

Zacharis et al (9) used this cold stage attachment in an X-ray diffraction study of the low-melting samples of an homologous series of highly purified mixed triglycerides. Where there is limited need for X-ray diffraction scans at lower temperatures and the temperature range provided is adequate, this inexpensive and easily fabricated attachment is a satisfactory substitute for the commercial cryogenic unit.

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[Received December 29, 1975]