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DSC AND HIGH RESOLUTION X-RAY DIFFRACTION COUPLING

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Coupling of time-resolved synchrotron X-ray diffraction at both small and wide angles with differential scanning calorimetry is a new technique that allows simultaneous characterization of thermal and structural properties of a sample. The apparatus, called Microcalix, works between –30 and +230°C at scanning rates comprised between 0.01 and 20°C min^{-1} with a high sensitivity in both measurements using a single sample of small volume (from about 1 to 20 μL). The last version of the instrument is designed for laboratory bench and conventional source but preferably with rotating anode or multilayered mirrors. Measurements under low pressure or under shear as well as recordings of isothermal evolution are also possible. The example of the study of polymorphism of a monounsaturated triglyceride (PPO) will be presented as an application.

Keywords: *lipid structures, mesophase and biomaterial studies, polymorphism, small angle X-ray scattering, triacylglycerol*

Introduction

The structural changes of materials are associated to modifications of the thermodynamical parameters which characterize them. Techniques most frequently used for the study of the structural modifications and of these thermodynamical parameters characterizing a system are generally and respectively those of X-ray diffraction and of thermal analysis. Whatever the brilliance of the X-ray source employed, from sealed tube to the brightest synchrotron, diffraction allows to determine material organizations at the atomic, molecular and supramolecular scales according to its degree of order and depending on the instrument used by the measurement of correlation between diffracted waves.

On the other hand, microcalorimetry is capable of determining with a high sensitivity, the thermal exchanges associated to the modifications of this organization. Monitoring of the energies exchanged between a material sample and its environment in thermally insulated conditions allows to determine its specific heat variations as a function of time and temperature. Scanning calorimetry allows the measurement of thermal parameters such as temperatures, T, and variations of enthalpies, ΔH, and entropies, ΔS, of phenomena associated to structural evolutions. However, both types of characterizations are generally obtained on independent apparatus operated by different operators what does not facilitate the correlation between both types of phenomena.

Differential scanning calorimetry (DSC) allows the characterization of the changes of state and more generally the reversibility of the phenomena involved in the phase transitions between condensed states. However, this technique does not inform on the structure existing before and after the phase transition recorded. The characterization of these transitions is often complicated by the existence of a polymorphism (capability of a substance to be crystallized under various different forms). Even in the absence of polymorphism, these transitions are always controlled by the time and temperature parameters making DSC a powerful instrument for the characterization of structural evolutions.

A differential microcalorimeter allowing both types of analysis simultaneously has been developed within the CNRS group, UMR 8612, first to be used on the benches D22 and D24 of LURE [1]. Several versions of this equipment were now realized and used on various synchrotrons for several years. The purpose of this paper and of the associated lecture is to illustrate the capabilities of this instrument and its easiness of use by showing some examples of thermal and structural analysis. The example chosen for this paper which is the polymorphism of a pure triglyceride can be easily transposed to more complex systems such as found in Nature. More information about the use of MICROCALIX for different systems is found below [2–9].

Experimental

PPO (1,2-dipalmitoyl 3-oleoylglycerol) (99%) was synthesized by Pierre Villeneuve in collaboration with Jean Graille (CIRAD, Montpellier) and used as such.

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XRDT/DSC measurements

The general scheme of coupling is shown Figs 1 and 2. This set up allows, in its last version, thermal scans in a T range $-30 \leq T \leq +230$°C, with scanning rates $0.01 \leq s \leq 5$°C min^{-1} (and up to more than 20°C min^{-1} depending on T range) and with a sensitivity comparable to that of modern commercial apparatus >100 μV mW^{-1} (Figs 3–5). Scanning temperature is controlled with a resolution set either at 0.001 or 0.01°C thank to two devices controlling temperature (REG) and (TCC).

Coupled X-ray diffraction (XRD) recorded at both small and wide angles and high-sensitivity DSC set-up was installed successively on both D22 and D24 benches of DCI synchrotron of LURE (Laboratoire pour l'Utilisation du Rayonnement Electromagnétique) in Orsay (France). This set-up has already been described [1, 3–5]. Briefly, on D22 bench, two position sensitive gas linear detectors (LD1 and LD2) allow the recording of simultaneous small (q=0–0.45 Å^{-1}) and wide (q=1.1–2.1 Å^{-1}) angle of XRD data with sample to detector distances of 177 and 30 cm, respectively. Both XRD data and DSC signals are simultaneously collected from the same sample with a single computer (PC comp.) in order to avoid any time or temperature shift in the data collection. This last are coming from both counting Electronics (counting Elect.) and from microcalorimeter head (CALO) through a microvoltmeter (mVmeter). On D24 bench, a single linear detector has been used at small angles (q=0–0.55 Å^{-1}) with a sample-to-detector distance of 90 cm. The channels of the detectors are calibrated to express the XRD data in scattering vector q with $q=4\pi\sin\theta/\lambda=2\pi/d$; q in Å^{-1}, θ in degrees is the angle of incidence of X-ray relative to the crystalline plane, λ is the X-ray wavelength, d in angstroms is the repetition distance between two planes [10]. The calibration of the detectors is made at wide angles with high-purity tristearin [4] complemented at small angles with silver behenate [11] standards, as previously described [3, 7, 8]. The calorimeter coupled to X-ray diffraction is calibrated with lauric acid [12].

Figure 2 shows the electronics of the microcalorimeter which is arranged in the foreground in front of the cryostat (TCC). The computer which is not presented here pilots the calorimeter through a line RS232C and through a bus IEEE-488. The line of diffraction of the X-rays arranged on a granite optical bench is visible in the top of the photo. On this beam line, from left to right, we distinguish successively: a monochromator, a set of vertical and horizontal slits all under vacuum, the microcalorimeter, a large vacuum tube containing the beam stop made of tungsten and a 200 mm long gas position sensitive detector.

Figure 3 shows a schematic cut of a calorimeter head. The two capillaries reference (ref.) and sample (sam.) are located in this figure in the same vertical plane. Peltier effect modules are used for temperature control of calorimeter body (C) in which capillaries are located. Capillaries used as sample-holders for both X-ray diffraction and DSC are 1.4±0.1 mm of diameter and about 80 mm long. The sample capillary is filled on about h=15 mm by the sample thank to a syringe specially developed for sample filling. The resulting volume represents usually about 20 mg, but different masses ($0.5 \leq m \leq 40$ mg) can be used [1]. This syringe allows filling with sample of viscosity ranging from liquid to soft solids.

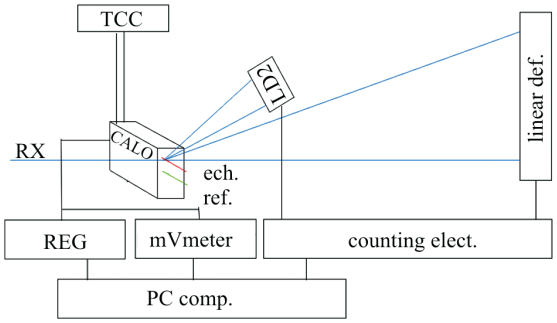


Fig. 1 Schematic drawing of Microcalix setup

Fig. 2 Photo of one of the calorimeters installed on the bench D24 of LURE with a single long position sensitive detector

Results and discussion

The calorimeter described above allows the study of thermal phenomena dependent on time and/or temperature such as those generated by polymorphism of materials. The study of polymorphism is of special interest for pharmacy since numerous pharmaceutical molecules display this property. We present below briefly an example of study realized on Microcalix with a pure triacylglycerol (99%) (formerly named triglycerides). A more detailed study of the polymorphism of PPO and the formation of the various crystalline forms of this compound is in the course of preparation and will be later published.

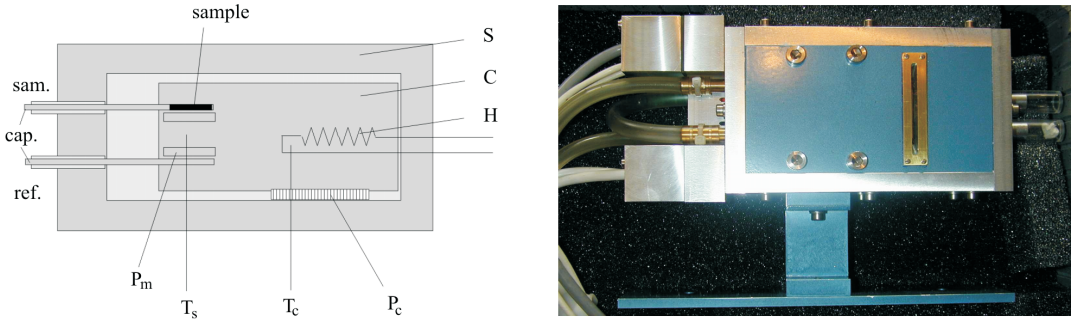


Fig. 3 Left – schematic cut and right – photo of a differential calorimeter. Several series of Peltier modules P_c, P_m control the calorimeter temperatures T_c and T_s at ±0.001°C thank to a heating device (H) as well as allows its measurement. A thermal screen surrounds the calorimeter

Polymorphism of PPO (1,2-dipalmitoyl 3-oleoylglycerol)

As most of the lipids, PPO presents a complex polymorphism of monotropic type due to the various possibilities of arrangement of the hydrocarbon chains in the solid-state or in liquid crystalline states. However, for the very weakly polar triacylglycerols such as PPO (this triglyceride is a constituent of fats), the polymorphism is less complex than for more polar lipids, because these lipids are essentially thermotropic substances. Lyotropic substances like most of polar lipids display more complex polymorphism since this last depends on water concentration [13].

As a consequence of this monotropic polymorphism, the cooling of PPO from melted state, at 65°C, down to 0°C of a sample about 20 mg of pure PPO (99%) at the rate of 5°C min^{-1} leads to the crystallization of a metastable crystalline variety. On heating, this metastable form will transform into more stable varieties. The recordings of differential microcalorimetry (DSC) and the evolutions of X-rays diffraction patterns observed at small and wide angles on heating at 1 K min^{-1} from the same sample are presented, respectively, in Figs 4 and 5 and decompose into four steps easily identified on both recordings as well as in Figs 6 and 7. Four different events three endo- and one exothermic are clearly visible in the domain 20–40°C. These transitions correspond to the structural changes evidenced Fig. 5 in which four types of structures which are clearly visibles in the domain 0–50°C, (delimited by double arrows) are delimited by the transitions evidenced Figs 4, 6 and 7 (dashed lines). In this figure, the line corrresponding to order 2 of the structural period of 75 Å is volontarily cut at half height to allow a better visualization of other lines at higher temperatures. In Fig. 7, the metastable structure initially formed with a period of about 75 Å (only order 2 line at 37.6–37.7 Å is shown on figure) transforms into a mixture of two forms, one with intermediate stability and a stable one of smaller periods (close to 33 and 40 Å) melting one after the other (vertical dashed lines delimit the domain of existence of phases observed).

DSC recordings display several thermal events. While the beginning of an endothermal event is first observed at about 20°C, it is partially masked by the fast development of an exothermal one overlapping the second moiety of the endotherm. In fact, both events occur almost simultaneously and self inhibit each other partially. As a consequence, in the intermediate part of this double transition, between maximum of both peaks, only the resultant of both phenomena is recorded. Such behavior is frequently observed in monotropic systems during melting of metastable crystalline forms when more stable nuclei formerly entrapped in this metastable variety are suddenly allowed to grow at the expense of the metastable variety according to a reaction.

metastable form→more stable form

The whole process being exothermic is only limited by the growth rate of more stable form. Here, the more stable crystalline form is an intermediate stability form. Note: The authors of this paper have shown that these monotropic transitions are liquid mediated but there is still strong debates about this view which is not accepted by the whole lipid structure community [14, 15]. This intermediate form more stable than the initial one in turn melts between 27 and 30°C (Figs 4 and 5) and is also transformed into more stable variety. The mechanism of the first reaction is rather simple and is schematized Fig. 8, however that of second is more complex.

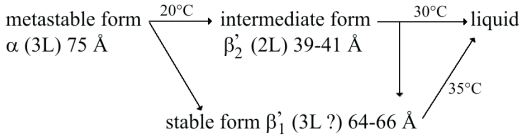
Only the quantification of evolution of the line intensities corresponding to the long spacings, measured as peak surface areas, allows to interpret the second transition. Figure 6 shows the evolutions of these surface areas according to the temperature for the three forms coexisting in the 20–30°C range, while Fig. 7 shows the evolution of their periods. In fact, the mechanism of transition is similar to the precedent one except that no exothermic event is recorded. The intermediate variety disappears at about 30°C to the benefit of the most stable variety

which only melts at about 35°C. Contrary to what was previously recorded for the first transition, both initial melting endotherm of intermediate stability form and recrystallization exotherm of the most stable variety are partially masked by their mutual overlappings. Figure 6 shows clearly that around 30°C a moiety of the crystals corresponding to the most stable variety results from the transformation:

intermediate stability form→stable form

The data recorded at wide angles allow to clarify that the metastable variety is of type α while the 2 more stable varieties are of type β' (meaning that the lateral packings of the hydrocarbon chains are respectively hexagonal or perpendicular orthorhombic) (Fig. 8). In this figure, the hydrocarbon chains of triglycerides symbolized by ·· (top drawing) or a line (bottom drawing) as they are seen from chain end or side. The chains of fatty acids are either vertical relatively to bilayer planes and arranged in a hexagonal lateral packing (left) or laterally packed in an orthorhombic perpendicular way while the fatty acid chains are tilted. The transition from a variety to a more stable one is translated by a volumic contraction and a release of heat. In X-ray diffraction, the α and β' forms display characteristic lines a single line at 4.15 Å for α and lines at 4.2 and 3.8 Å for β'. These lines and a transition α→β' are very clearly visible on Fig. 5 (WAXD part).

The weak line observed at small angles towards q=0.08 Å^{-1} (75 Å) becoming sharper on heating, is interpreted as the order 1 of the line at q=0.16 Å^{-1} (37.5 Å). This distance can only be identified with a stacking of at least three vertical chain lengthes (noted 3L). As a summary, the coupling of DSC with DRXT allows the quantitative interpretation of the DSC recordings and the identification of the following transitions.



The period of 75 Å observed for the form α (3L) is in good agreement with the distances observed for other triglycerides (triacylglycerols made from palmitic acid, P, stearic acid, S, and oleic acid, O) PPP, SSS and SSO crystallized in form α (2L or 3L), respectively with periods 45.6, 50.7 and 85 Å. The distances observed for the varieties β'_2 and β'_1 indicate that chains are tilted with respect to the layer planes and correspond to 2L stacking (about 40 Å) and possibly 3L (env. 65 Å) although the order 1 of this period is not visible. The intensity variations of the lines of forms β'_2 and β'_1 shown Fig. 6 demonstrate that their coexistence is due to their respective growth rates. The form β'_2 transforms first, but its growth rate is not too small to allow a full conversion of the liquid issued from the melting of α leaving space for form β'_1 to grow in turn. Ultimately, the melting of form β'_2 will allow a further growth of form β'_1 (Fig. 6).

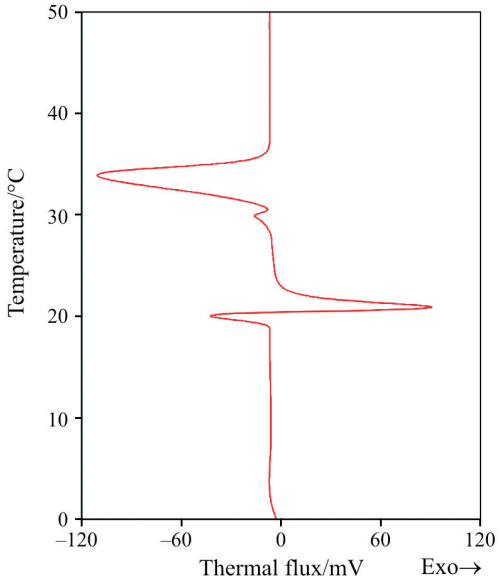


Fig. 4 DSC signal recording observed on the heating from 0 to 50°C at 1°C from a sample of about 20 mg of PPO, a monounsaturated triglyceride

This DSC equipment is currently used by several scientific laboratories and industrial partners for the characterization of the thermal and structural properties of very numerous compounds, mixtures, manufactured goods in mono- or poly-phasic systems. It has shown its usefulness in domains of chemistry and biology from food industry to pharmacy. Hundreds of thousand diagrams of diffraction corresponding to lipids (phospholipids, fatty acids fat, soaps, waxes, surfactants, emulsifiers, glycerides, alcohols, etc.), polymers, proteins, peptides, nucleic acids, were recorded with thousands of DSC recordings corresponding to the thermal evolution of products in different forms such as solutions, micellar solutions, dispersions, liposomes, emulsions, pastes, gels, powders, etc. These products are used in pharmacy as excipients or active principles, in the food industry (cream, milk, cheeses, chocolate, biscuits, starch products, foams) in cosmetic (lipstick, emulsions, skin and stratum corneum), chemistry (oil production, parafines, chlathrates, cyclodextrins, crystallization of water, etc.).

For several years, the various prototype versions of Microcalix were put at the disposal of all the users of benches D22 and D24 within the framework of scientific collaborations. They are at present available at the small angles bench BL 5.2 of the synchrotron

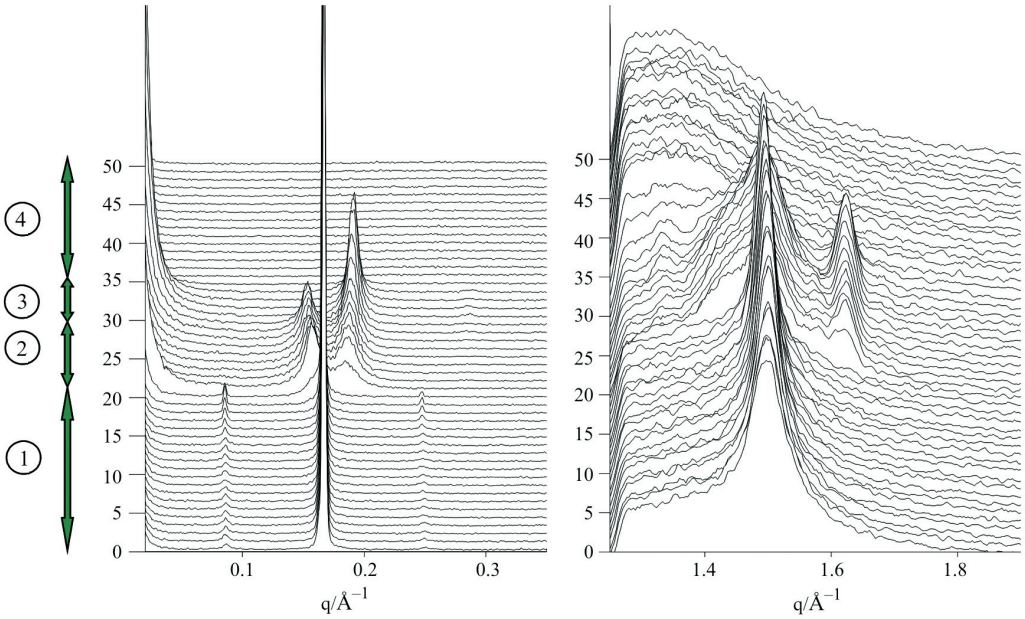


Fig. 5 3D representation of the evolutions of diffraction patterns observed at left – small and right – wide angles shown as Intensity as a function of scattering vector q and temperature T during the heating of sample of Fig. 4. The four types of structures which are clearly visibles in the domain 0–50°C, (delimited by double arrows) are delimited by the transitions evidenced Figs 4, 6 and 7 (dashed lines). The line corrresponding to order 2 of the structural period of 75 Å is volontarily cut at half height to allow a better visualization of other lines at high temperature

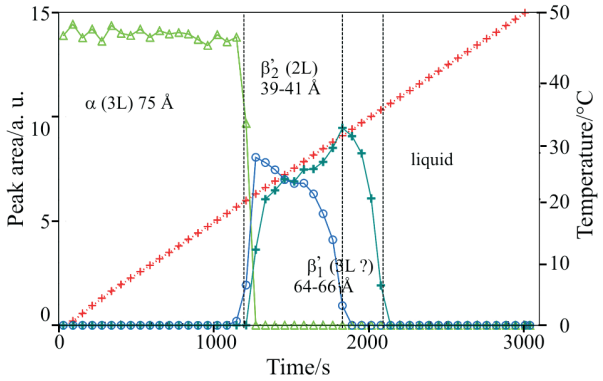


Fig. 6 Evolution of the surface areas of the peaks observed at small angles (Fig. 5a) corresponding to long spacings (periods of bilayered lamellar phases) of the various polymorphic species of PPO, shown Fig. 7, during sample heating at 1°C min^{-1}; ∆ – the unstable structure initially formed is transformed at about 20°C into a mixture of a o – form of intermediate stability and + – a stable form which melt one after the other (vertical dashed lines delimit the domain of existence of phases observed)

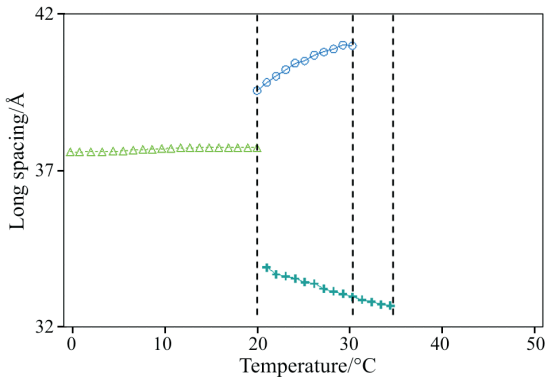


Fig. 7 Evolution of the interplanar distances of the various polymorphic varieties of PPO corresponding to the lines observed at small angles (Fig. 5a) the surface area evolutions are shown Fig. 6 as a function of time and temperature, during sample heating at 1°C min^{-1}

Elettra of Trieste (Italy [16]. It will be available soon at the experiments SWING of SOLEIL (Gif/Yvette) in 2006 and later at LEMAX [16], a diffraction line mainly dedicated to the industry experiments.

A new version of the calorimeter with vertical samples has just been finished [7, 17]. It was recently tested at Elettra and allows X-ray diffraction under shear at chosen constant shear rates [17]. Modulated DSC allowing recordings at scan rates as low as 1 K day^{-1} have been performed successfully. A new laboratory version of this DSC with both simultaneous small and wide angles recordings is under development in our group. Attempts are in progress to bring this instrument to a point where he can be marketed by companies building equipments of diffraction of X-rays.

In conclusion, the prototypes recently developed of Microcalix allowed the study of very numerous samples and demonstrated the power of coupling of X-ray diffraction with differential microcalorimetry for the investigation of properties and structures formed in complex systems such as those found in biology or in

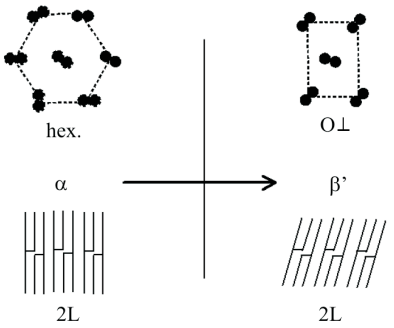


Fig. 8 Schematic representation of a transition α→β' for triglycerides

manufactured products. Microcalix, is a new instrument which allows the simultaneous physical characterization of the thermal and structural properties of materials on the same sample of small volume directly during a temperature scan or in isothermal mode. The current apparatus allows using synchrotron radiation the study of materials with a very high sensivity for both techniques. Evolutions of the structures and of thermodynamical properties of a single sample of small volume (about 20 μL) in the T range $-30 \leq T \leq +230$°C at scanning rates $0.01 \leq s \leq 20$°C min^{-1} and at sample pressures p, $1 \leq p \leq 10$ bars [16].

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